GENETIC STUDIES OF ASCITES IN BROILER POPULATIONS

Pau Navarro-Martinez

Thesis submitted for the degree
of
Doctor of Philosophy

The University of Edinburgh

2003

DECLARATION

I declare that this thesis has been composed by me. It is mainly the product of my own work and contributions from others are acknowledged within.

ABSTRACT

Continuous genetic improvement of growth and conformation traits in broiler populations has coincided with an increase in defects in heart and lung function. These defects have led to an increased incidence of metabolic disorders such as ascites (or pulmonary hypertension), a functional hypoxia. The incidence of ascites in well-managed flocks is low, but it nonetheless causes important economic losses to the breeding industry and is an important issue from a welfare standpoint. The aim of this thesis was to study the genetics of ascites-related quantitative traits.

A low blood oxygen saturation (SaO) value is a good indicator of ascites susceptibility. The existence of substantial genetic (polygenic) variation for SaO was demonstrated for four meat-type chicken lines. Estimates of heritabilities for SaO ranged from 0.1 to 0.2 and additive genetic correlations with production traits were not different from zero. SaO data from one of these lines were analysed using a mixed inheritance model (i.e., including a major locus and polygenes) and the results suggested that a major locus with two alleles at intermediate frequencies affected SaO. The putative major locus accounted for a difference of 13 % SaO between homozygotes and the decreasing allele was recessive. The major locus was also estimated to have an overdominant effect on weight and fleshing score. The mode of action of the putative major locus on SaO and production traits would hinder manipulation of its allele frequency without the use of molecular markers.

A population was designed to map this putative major locus. Power studies were performed to select a number of sires and their half-sib progeny. Sires were selected on the basis of their probability of being heterozygous at the putative major locus as estimated by the segregation analysis. Regions around the three ryanodine receptor loci (RYR1, RYR2 and RYR3), which are candidate genes for ascites, were chosen to perform a linkage study. No evidence of linkage of any of the regions studied with SaO, as a predictor of ascites, was detected. However, strong evidence of a quantitative trait locus for fleshing score linked to the RYR3 region was found. The resource population created for this study could be used to

test other candidate regions or a genome scan could be carried out to provide broader vision of genomic regions that control SaO and the ascites syndrome.

The study of an F_2 population derived from the cross of a broiler and a layer line with phenotypes on a series of traits that are known to vary in birds that suffer from heart, lung or muscular dysfunction, more commonly suffered by broilers than layers, revealed several genomic regions that affect these health related traits and that merit further study.

ACKNOWLEDGEMENTS

I would like to thank Chris Haley and Peter Visscher for supervising this thesis. Their comments and suggestions, together with discussions have always been helpful. I am grateful for support, patience and encouragement from my arrival to Edinburgh.

This project was the product of a BBSRC Industrial CASE studentship awarded to Aviagen Ltd. Aviagen Ltd provided the majority of the data analysed as well as financial support and I would like to show my appreciation for this.

Many people were involved in this project (from data collection to genotyping) and have helped to make it possible. Amongst them, I would like to thank especially Alfons Koerhuis, Dimitrios Chatziplis, Stewart Brown and David Harry (from Aviagen Ltd.) for their input. Thanks are also due to Paul Hocking and Dave Burt for providing data analysed in chapter five and Jacqueline Smith for guidance during my brief incursions at the lab.

Many colleagues have been a great help throughout and for that I am thankful. Andrew George and Ricardo Pong-Wong were always available for discussions. Ricardo provided, in addition, computer programs that were used in chapter three. Jules and Dimitris were there for help and "general comforting" and let me "steal" their food whenever the need arose. Heli and Maria Jose shared their home with us while our flat was being decorated. Ian White, Sara Knott and Sue Brotherstone have helped out in many ways and many occasions. Ian has tried hard to make me understand concepts that I found somehow obscure and has been of great assistance in making me "love and understand" ASREML. Sara has always listened to my not-always-ordered thoughts and our discussions have helped me make sense of them. Together with Chris, she provided the FORTRAN programs used (only with slight modifications) in chapter five. I need to thank Sue for being "the source" of tips and tricks relating to UNIX machines.

I have intensively used computer resources both from Roslin Institute and the University of Edinburgh, and would like to thank both IT teams for providing good

resources. QTL Express has also been used intensively, and George Seaton has kindly listened to suggestions regarding output availability and format.

On a personal note I would like to thank Beatriz, Geoff and Marie for friendship.

A very big "thanks" and many hugs go to my mum and dad that have always been there for me and to my sister. I am sure they will all join me to thank Alexander Graham Bell for inventing his "electrical speech machine". I would also like to thank friends and "extended family", especially Karla and Valentin for "sticking" with us. My dad will be glad if I take this opportunity to put in writing a thought that has crossed his mind more than once: "What's the problem with having Roslin Institute and the University of Edinburgh moved to Valencia?".

Of course, I am thankful to Albert for many years of friendship and love, and for taking up the challenge of building our life together.

PUBLICATIONS

The following publications have resulted from the research described in this thesis:

Navarro P, Koerhuis ANM, Chatziplis D, Visscher PM & Haley CS (2001). Does a major gene control ascites susceptibility? 13th European Symposium of Poultry Nutrition, Blankenberge (Belgium), pp. 336-337.

Navarro P, Koerhuis ANM, Chatziplis D, Visscher PM & Haley CS (2001). Genetic studies of ascites in a broiler population. 2nd European Poultry Genetics Symposium, Gödöllő (Hungary), pp. 106-107.

Navarro P, Koerhuis ANM, Chatziplis D, Visscher PM & Haley CS (2002). Genetic studies of ascites in a broiler population. 7th World Congress on Genetics Applied to Livestock Production, Montpellier (France). CD-ROM communication n° 04-14.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

-2LnLR	-2 times the logarithm of the likelihood ratio
7dwt	body weight at seven days of age
a	additive effect
ACTN2	actinin, alpha 2
ADPRT	poly(ADP-ribosyl)transferase
API	arterial pressure index
BAC	bacterial artificial chromosome
BMPR-2	bone morphogenetic protein receptor, type II
c^2	ratio of maternal environmental variance to total variance
Ca	calcium
Ca^{2+}	calcium bivalent cation
CAPN1	calpain 1, large subunit
CARCASS	Dressed Carcass weight
cM	centiMorgan
d	dominance effect
DGAT1	diacylglycerol O-acyltransferase homolog 1
DNA	deoxyribonucleic acid
ECG	electrocardiogram lead S-wave amplitude
FISH	fluorescent in situ hybridasation
Flesh	fleshing score
g^2	ratio of maternal genetic variance to total variance
Gain	weight gain between seven days and five weeks of age
GGA	chicken chromosome
GIZZARD	gizzard weight
h^2	heritability
HEART	heart weight
HSA	human chromosome
i	"imprinting" (parent-of-origin) effect
IC	marker information content
IGF2	insulin-like growth factor 2
INS	proinsulin precursor
INTESTINE	intestine length
LAD	Leukocyte adhesion deficiency
LG	linkage groups
LIVER	liver weight
LNCREAT	Ln-transformed creatinine kinase concentration

LNTROP	Ln-transformed troponin T concentration
MAI	marker assisted introgression
MAS	marker assisted selection
MAX	maximum
Mb	Mega base
MCMC	Markov Chain Monte Carlo
MCV	mean cell volume
MIN	minimum
MYOD1	myogenic factor 3
O_2	oxygen
p_b	frequency of the b allele
p_B	frequency of the B allele
p_{bb}	frequency of the bb genotype
p_{Bb}	frequency of the Bb genotype
p_{BB}	frequency of the BB genotype
PCR	polymerase chain reaction
PCV	packed cell volume
PH	pulmonary hypertension
PO_2	partial pressure of oxygen
QTL	quantitative trait locus/loci
REML	restricted maximum likelihood
RFLP	restriction fragment length polymorphism
r_g	genetic correlation
r_p	phenotypic correlation
RN	Napole yield
RYR	Ryanodine receptor
RYR1	Ryanodine receptor 1
RYR2	Ryanodine receptor 2
RYR3	Ryanodine receptor 3
SaO	blood oxygen saturation
SD	standard deviation
SE	standard error
Selwt	selection weight
SPLEEN	spleen weight
T3	triiodothyronine
T4	thyroxine
TBCC	total blood cell count
TGF-beta2	transforming growth factor-beta 2
TGF-beta3	transforming growth factor-beta 3

TH	tyrosine hydroxylase
Weight	body weight
σ_e^2	residual variance
σ_{em}^2	maternal environmental variance
σ_{gm}^2	maternal genetic variance
σ_m^2	major locus variance
σ_p^2	phenotypic variance
σ_{QTL}^2	QTL variance
σ_u^2	genetic (polygenic) variance

CHAPTER ONE

1 GENERAL INTRODUCTION

1.1 Background on the broiler breeding industry and its present challenges

Worldwide meat consumption from farm animals has been growing regularly during the last decades. In 1999, poultry meat production represented 29 percent of total meat production (this is around 40 million tonnes out of 140) and this proportion is likely to have a steady yearly increase in the future, because the growth of poultry meat consumption is the result of a strong consumer demand for products perceived as affordable, safe and healthy (McKay *et al.*, 2000). Poultry meat production will need to increase in absolute terms if it is to meet market demand.

Broiler breeders are continuously seeking to increase production through genetic improvement of their stock and adaptations of nutrition and management programmes. At the same time, they want also to improve economic efficiency.

The income from the sale of a broiler chicken depends on its weight, but the price per unit weight depends, at least in some markets, on the composition of the animal. For instance, fat content and the proportion of the weight as the more valuable anatomical parts, mainly skeletal muscles and particularly breast muscle (that is the most valuable part of the carcass) have an impact on the price per unit weight. Profit, nonetheless, depends not only on income, but also on the costs of getting the animal to its market weight and quality (Emmans and Kyriazakis, 2000).

The major costs in the chicken industry are associated with feed and the time it takes to the animal to reach its market weight (Van Horne, 2000). A way to decrease this cost is to select those animals that reach the commercial weight quicker and have a smaller cost of feed. This is why poultry breeders have selected their stock mainly on growth rate for generations. Selection has also been done on feed conversion efficiency and, most recently, meat yield and/or yield of breast meat in order to obtain animals that give a greater profit per unit.

As a result of continuous selection on these traits and improvement of nutrition, management techniques and environment growth rate has increased almost in a linear fashion (Classen, 2000). MacKay *et al.* (2000) estimated that from the early 1980s this linear increase has been of around 60 grams per year. Studies from the early 1990s (*e.g.* Fairfull *et al.* (1998), Havenstein *et al.* (1994)) claimed that the 1990s broiler grew more than three times faster than poultry meat stock in the late 1950s, had a better feed conversion, which made it reach the market weight with less than half the feed, and had a greater carcass yield. Havenstein *et al.* (1994) estimated that genetic improvement was mainly responsible of these changes (83.3 percent for growth rate, 62.5 percent for feed conversion and 91.3 percent for carcass yield) with the remainder being due to the diet. More recent studies (Chapman *et al.*, 2003; Havenstein *et al.*, 2003a; Havenstein *et al.*, 2003b) show that great improvements are still achievable and that in 2001 a typical 42-day old broiler fed on a typical 2001 diet was almost five times as heavy as a 42-day old 1957 broiler fed on a 1957 diet. Feed conversion has also continued to improve, and the same target weight can be attained in a third of the time with a three-fold reduction in feed; at the same time, breast meat yield has increased comparatively more than yield of other carcass parts. The improvement is mainly due to genetic selection and has taken place in the last ten years.

Rates of annual progress achieved for some production traits are presented in Table 1.1.

Table 1.1. Mean values of some characters of economic interest and rates of yearly progress for male broiler chicken.

	Current Values	Yearly Progress	
		Absolute values	Rates (%)
Food conversion ratio	1.66	(-)0.02	1.2
Weight at 42 days (g)	2600	60	2.4
Breast meat yield (g)	460	17	1.4

Adapted from McKay *et al.* (2000).

Several authors have pointed out that long term selection on growth and conformation related traits has not been without undesirable consequences such as reduced reproductive performance (through both reduced fertility and egg production (Dunnington and Siegel, 1996)), decreased viability (Emmerson, 1997; Havenstein *et al.*, 2003b) increased incidence of defects in skeletal development and heart and lung function (Emmerson, 1997; McKay *et al.* 2000), as well as other support systems (digestive and excretory) and decreased adaptability to environmental conditions (Rauw *et al.*, 1998; Emmans and Kyriazakis, 2000). Cheema *et al.* (2003) also noted changes in immune response in modern broilers when compared with 1957-type ones. Following the "resource allocation theory" (Beilharz *et al.*, 1993), Rauw *et al.* (1998) suggest that when a population is driven to high production by artificial (genetic) selection, less resources will be available to respond to other demands, and that this would most likely affect traits not explicitly taken into account in the breeding goal. Decreased reproductive performance, increased health problems and reduced viability not only increase the cost of producing commercial chicks (Rose, 1997) but also hinder further progress on growth and conformation traits and have a negative impact on broiler welfare. Changes in nutrition and management programmes can partly alleviate these problems. For instance, feed-restriction of reproductive stock is, nowadays, common practice since reproductive problems are increased by excess fatness (Fairfull *et al.*, 1998). Nonetheless, if fitness and health traits could be improved through genetic selection, progress would be most durable since it would be transmitted from generation to generation. In recent years, the broiler breeding industry has shown a will to improve health related characters and produce more robust birds. Breeding programmes have been and are being adapted to quickly respond to adverse genetic correlated responses to selection on production traits (McKay *et al.*, 2000) and, for example, during the last decade the incidence of leg problems seem to have decreased.

Rapid growth and economic efficiency are still, nonetheless, the main priorities for the poultry meat industry, and its aim is to sustain the increase in growth rate that has been

achieved in the last 50 years. Although there is still genetic variation for production traits (see for example, Koerhuis and Thompson (1997)), this will, however, not be possible if the physiological equilibrium of broiler stock is disrupted, so care must be taken in controlling undesired correlated responses of health related traits to selection on production traits. Knowledge of the genetic basis of production and health traits and their relationships is therefore necessary to achieve optimal progress on production traits without disturbing the birds well being and capacity to adapt to a variety of environments.

The first three results chapters of this thesis are devoted to the study of the genetics of ascites (or Pulmonary Hypertension (PH)) and its genetic relationship with growth (live weight) and fleshing score, that gives an indication on breast conformation, in meat-type chicken breeding populations. Ascites is a complex metabolic disorder observed in many species, but most commonly seen in males of fast-growing lines of broilers (Squires and Summers, 1993).

In chapter five we present results from an analysis of data from an F_2 population derived from the cross of a broiler and a layer lines to look for genomic regions involved in the control of health-related traits, focusing on disorders that most frequently affect broilers, such as cardiovascular and muscular disorders.

1.2 An overview on the ascites syndrome in modern broiler stock

A wealth of studies on the different aspects (causes, management to reduce its incidence, pathophysiology, etc.) of the ascites syndrome is available on the literature. We have consciously restricted our review, focusing on the aspects that are most relevant to our study. We first describe briefly the disorder in modern broilers and its importance to the industry. We then give an overview of recent work that provides a vision of possible strategies to decrease ascites incidence through selection in broiler stock.

The presence of ascites is characterised by the accumulation of fluid in the peritoneal cavity and that makes that the disorder in also known as "waterbelly". Julian (1993) states that "ascites is a sign or lesion that may result for one or more of four physiological changes

that cause an increased production or decreased removal of peritoneal lymph". The causes of ascites may be:

1. Obstruction of lymph drainage
2. Decreased plasma oncotic pressure
3. Increased vascular permeability, caused by endothelial damage
4. Increased hydraulic pressure in the blood vascular system.

In modern broiler stock, ascites is more often a consequence of 4. Ascitic birds were first observed in flocks reared at high altitudes (Maxwell *et al.*, 1986), where the oxygen tension in the air is low, but since the 1980s, the disorder has been observed also at low altitudes, even at sea level, most frequently in males. Ascites can lead to the death of affected birds and has also been linked to the incidence of heart-related sudden deaths (sudden death syndrome or flip-over) (Squires and Summers, 1993; Olkowski and Classen, 2000). During the 1980s and 1990s, ascites–related mortality steadily increased in the Netherlands (Scheele, 1996). Nowadays, worldwide ascites-related mortality has been estimated to be around 3.8 percent (Maxwell and Robertson, 2000). Although a variety of environmental factors (*e.g.* high altitude, low temperature), diseases, nutritional regimes, management practices, toxins, etc. (Mitchell, 1997) are known to trigger the condition, it is still observed in flocks where these factors are at near optimum levels. Because ascites incidence in broiler populations has increased together with performance in production (*i.e.*, growth and conformation) traits, most of which was due to genetic selection (Havenstein *et al.*, 1994), it has been tacitly accepted that ascites was linked to it and that it was somehow under genetic control. Specifically, Julian (1993) postulated that the ascites syndrome in modern meat-type chickens reared at low altitudes and in commercial conditions was related to the high oxygen requirement of rapid growth and the inability of the heart and lung to deliver sufficient oxygen to the tissue to maintain the growth potential of the animal. This arises because of the greatly increased growth rate at a given weight, and although requirements for maintenance are relatively low in genotypes growing rapidly, their absolute requirement of energy per day

is substantially increased, and so is daily intake (Dunnington and Siegel, 1996). As intake increases, so does the oxygen requirement to oxidize the substrates and the respiratory system of the bird needs to work harder to satisfy this increased oxygen demand. However, modern broiler stock lacks an efficient respiratory system since continuous selection for increased live weight at relatively young ages has not only affected the growth curve of broilers over the past 50 years by increasing growth rates dramatically, especially at early stages -making birds that are less mature when they reach the slaughter weight- (Emmans and Kyriazakis, 2000), but it has also had an impact on the differential growth of organs (Dunnington and Siegel, 1996). Demand organs (muscles, skeleton, skin...) have experienced greater growth than supply organs (heart, lungs, gastrointestinal tract...), and modern stock has comparatively smaller lungs and heart than 1950s typical broiler stock (Havenstein *et al.*, 2003a). Complications due to smaller and more immature lungs have been accentuated by selection on breast yield, which has led to an increased proportion of the body skeletal muscle with respect to the total body weight (Emmans and Kyriazakis, 2000) and to a large breast-muscle mass, which interferes with respiration (Julian, 1993). The oxygen inhaled needs to be transported to the tissues where it will be used through the blood pumped by a relatively small heart that needs to respond to a high demand of oxygen and deliver more viscous blood (see next section) to the lungs that in turn have an insufficient vascular capillary capacity, which increases the strain put on the bird's heart. The increase in workload of the heart usually results in right ventricular hypertrophy (Julian, 1993) and subsequently the cardiovascular function starts to fail. As the heart fails, fluid accumulates in the abdominal cavity (Lubritz *et al.*, 1995) as typically observed in ascitic birds. Ultimately, this cascade of events may lead to the death of affected stock.

Although the incidence of the disorder in well-managed flocks is low, economic losses from ascites or heart failure are high since mortality occurs usually in birds close to market weight (Hunton, 1998). Maxwell and Robertson (1997) estimated that ascites caused losses of around one billion dollars per annum to poultry farmers through mortality in the

broiler house. This figure is further increased through condemnation of carcasses in the processing plant (Ross Breeders Ltd., 1996) that can be as high as 19 percent (Olkowski *et al.*, 1996).

1.2.1 Candidate ascites indicator traits

Ascites has been defined as a functional hypoxia caused by the inability of the respiratory and circulatory systems to provide the amount of oxygen needed by the fast growing modern broiler (Julian, 1993). It is a complex disorder that can be triggered by different factors that put extra strain mainly on the respiratory and/or cardiovascular systems and that provokes a cascade of physiological reactions in affected birds. There is still some controversy about what are, amongst a series of changes observed in ascitic birds, causative factors and what are effects of the disorder. For example, Olkowsky *et al.* (1999) claimed that although hypoxemia is associated with ascites in broiler chickens and that it also occurs in many apparently normal fast-growing broiler chickens, it is not clear whether it is a causative factor of the pathogenesis of ascites or a sign of heart failure.

We will give an overview of the most commonly observed changes and of those that could potentially be used as indicators of susceptibility to ascites in selection programmes aimed to reduce the incidence of the disorder, but will not discuss whether they are causative factors or consequences of the susceptibility to or presence of the ascites syndrome.

Maxwell *et al.* (1986) studied ascitic birds (*i.e.*, that presented excessive accumulation of fluids in the abdominal cavity) reared at low altitudes and described pathological and haematological changes in these birds compared to non-ascitic controls. They observed that ascitic birds were smaller, had relatively larger hearts than controls and suffered from right ventricular distension. In a wealth of studies reviewed (*e.g.* Lubritz *et al.* (1995), Wideman *et al.* (1998), Druyan *et al.* (1999), De Greef *et al.* (2001b)), hypertrophy of the right ventricle of the heart was consistently observed in ascitic birds, and the ratio of the right ventricle weight over the total ventricular weight –or arterial pressure index (API) of the heart- is a reliable indicator of the presence of the condition even before fluid

accumulation in the abdominal cavity occurs. Maxwell *et al.* (1986) also observed that liver, kidneys, lungs and intestines of affected birds were variable in appearance but, generally, they were all congested and the livers were shrunken, the kidneys enlarged, the spleens small and the breast muscle was darker in ascitic broilers compared with controls. Microscopic changes were also observed in all these organs but will not be reviewed.

Haematological analyses revealed as well differences between affected and non-affected birds. Amongst other changes, ascitic birds presented significantly increased haemoglobin, packed cell volume (PCV) or haematocrit and red blood cell counts and tended to have higher mean cell volume (MCV) and mean cell haemoglobin. Ascitic birds generally had more viscous blood than unaffected birds (Scheele, 1996).

The authors concluded that changes observed in low-altitude ascitic birds closely corresponded to the ones observed in earlier studies of ascitic birds that had been reared at high altitudes (*i.e.*, under low atmospheric oxygen tension). Latter studies (Maxwell *et al.*, 1990a) observed again milder but similar signs in birds subjected to experimentally induced hypoxia.

Julian and Mirsalimi (1992) studied blood oxygen saturation (SaO) (see Box 1.1) in ascitic and healthy slow or fast growing broilers. SaO is a measure of the blood oxygen content, which is related to the amount of this gas that could be supplied by the circulatory system to the cells. They showed that broilers suffering from ascites had lower SaO than fast or slow growing healthy contemporaries and that fast growing healthy birds had lower SaO than slow growing ones. Druyan *et al.* (1999) also found that ascitic broilers had low SaO levels.

Box 1.1. Blood oxygen saturation: definition and background.

Oxygen saturation (SaO) measures the amount of oxygen (O_2) chemically combined with haemoglobin in unit volume of the blood (Roughton, 1964). It is a measure of the blood oxygen content, which is related to the amount of this gas that could be supplied by the circulatory system to the cells and is defined as:

SaO = 100*(Actual O_2 content – O_2 in simple solution) / O_2 capacity of the blood.

A healthy individual should have a high SaO.

SaO depends on the partial pressure of O_2 (PO_2) with which the blood or haemoglobin solution is in equilibrium. The oxyhaemoglobin dissociation curve –also known as the oxygen dissociation curve and the equilibrium curve of the blood- describes this relationship (Roughton 1964).

Because ascites is a functional hypoxia (which could either be caused by poor oxygen supply or great oxygen demand), SaO could be used as an indicator of the disorder.

Maxwell *et al.* (1994) and Maxwell *et al.* (1995) showed that serum cardiac Troponin T, used in diagnosis of early myocardial damage in humans was increased in ascitic broilers from early ages.

Selection experiments carried out in some broiler populations to study ascites susceptibility have shown that in these populations this trait is partly under genetic control (see for example, Druyan *et al.* (2001), Druyan *et al.* (2002), Pavlidis *et al.* (2002), Wideman and French (1999) and Wideman and French (2000)), and that genetic selection against the disorder is possible.

In early stages of the disorder or for not severely affected birds, ascites presence can only be assessed in post-mortem examinations and, consequently, direct selection against it needs to be done using family information, with the costs attached to the procedure and the rearing of sibs of selection candidates, often in ascites-inducing (*e.g.* cold) environments. The availability of cheap and easy-to-measure traits that could be used as indicators of early presence or even susceptibility to ascites in live birds would represent a great advantage, especially from a breeder's perspective, since selection against ascites would be possible at a smaller cost.

In recent years, several authors have studied clinical and subclinical changes observed in ascitic birds and assessed their performance as indicators of (early) presence of the disorder or predictors of ascites susceptibility (as opposed to, for example, general stress or debility signs).

A good indicator trait to be used in commercial breeding programmes to select against ascites susceptibility needs to be, in addition to non-invasive, cheap and easy to measure, highly correlated with accumulation of fluid in the abdominal cavity and heritable. Ideally, for selection both against ascites and for performance traits, the genetic correlations of the indicator trait with ascites and performance traits would have opposite signs.

Wideman *et al.* (1998) studied the performance of a series of minimally invasive traits (body weight, heamatocrit values, heart rate, electrocardiogram lead S-wave amplitude (ECG) and SaO) to predict ascites susceptibility. They observed that weight at one or 14 days of age was not predictive of susceptibility to ascites but birds that were diagnosed as ascitic at 51 days of age were lighter at 42 days of age. They also observed that growth rate decelerated subsequent to the onset of ascites. Heamatocrit and ECG values of birds that went on to develop ascites (pre-ascitic) were higher than those of birds that did not, but SaO values of pre-ascitic birds were lower. The authors suggested the use of these traits combined in indices as diagnostic tools for breeding purposes after evaluating thresholds for each trait to distinguish birds prone to ascites from healthy flockmates. They advised that evaluations should be done within each population and under the same environmental conditions that breeding is to be carried out. On the same line, De Greef *et al.* (2001b) proposed an aggregate trait based on sub-clinical indicators of ascites (API, PCV, pericardial moisture score, score for dilation of right ventricle, heart fibrinogen and liver colour score) and showed that it performed well to predict ascites mortality in cold-housed broilers. The drawback of this aggregate trait was that some of its components cannot be measured non-invasively. The same authors suggest that blood gas traits could be attractive candidates to be used as indicators because their relationship with ascites mortality was consistent between

and within lines. Like Wideman *et al.* (1998), Roush and Wideman (2000) observed different patterns of growth between ascitic and non-ascitic broilers and Roush *et al.* (2001) showed that less than a week's worth of growth velocity data recorded on two-week-old chicks could be used to efficiently predict ascites susceptibility.

Heritabilities and genetic correlations with ascites and/or production traits have been estimated for some of these candidate indicator traits in some broiler populations mainly reared under cold stress to increase the incidence of the disorder. For instance, Lubritz *et al.* (1995) estimated heritabilities of between 0.1 and 0.4 for ascites in three broiler lines and of 0.2-0.3 for heart-API. They estimated a genetic correlation greater than 0.5 between the two traits, confirming that they were strongly related. Further studies (*e.g.* Druyan *et al.* (1999), Maxwell *et al.* (1998), De Greef *et al.* (2001a), Moghadam *et al.* (2001), Pakdel *et al.* (2002a), Pakdel *et al.* (2002b)) have shown that ascites or ascites mortality and some of these indicators (*e.g.* SaO, heart rate, ECG, heamatocrit) were also heritable in their populations. Their results will be reviewed and discussed in more depth in chapter 2. More importantly, Shlosberg *et al.* (1996) showed that selection for high heamatocrit increased ascites-related mortality and selection for low heamatocrit reduced it (although the low heamatocrit line had a higher total mortality), proving that selection on indicator traits can be effective in reducing susceptibility to ascites.

Amongst the candidate indicator traits, SaO can be measured easily and non-invasively by means of spectrophotometry using an oximeter, which is a device that uses this technique to measure the percentage of haemoglobin oxygen saturation in the blood. It does this "by measuring the amount of light energy lost in a tissue bed perfused by pulsatile blood" (Neoforma Inc., 1999). This allows quick SaO estimates. In humans, this device is used to continuously monitor SaO, by attaching a non-invasive probe to the skin of the earlobe or finger, usually during critical care and surgical procedures, but also in physiological studies (for example on athletes) (CDRH, 1997). It can also be used in livestock species, with some adaptations. In poultry the probe is attached either to the wing

skin or to the crest and allows easily recording SaO on large numbers of birds, which can include selection candidates, making it convenient to use in commercial breeding programmes. Low SaO values have been shown to reliably predict ascites susceptibility (*e.g.* Wideman *et al.*, 1998). Moreover, SaO is a continuous trait and therefore provides a continuous measure of susceptibility to ascites.

1.3 Aims of the study

During the past years, many studies have been done on the physiological causes and effects of ascites (see Currie, 1999 and Julian, 1993 for reviews) but, to date, little research has been carried out about possible genetic causes of the disorder. The aim of this thesis is the study of the genetics of ascites through the analysis of SaO, an indicator of susceptibility to this complex disorder. We will estimate the proportion of the variance observed for the SaO explained by genetic factors and its genetic correlation with production traits (body weight and fleshing score). Understanding the genetic architecture of this trait and its genetic relationship with production traits is necessary to implement a successful selection strategy that allows both sustained improvement of production traits and reduces the incidence of ascites in meat-type chicken populations. We are particularly interested in assessing the possible existence of a major gene or quantitative trait locus (QTL) involved in the control of SaO, and the ultimate aim of this study is the mapping of this major gene or QTL if it exists. To this end, we will analyse SaO data from a broiler population using a mixed inheritance model (including both a major locus and polygenes) and subsequently test genomic regions around the three Ryanodine receptors for linkage. These regions were chosen based on prior information that suggested the involvement of Ryanodine receptor 2 in right ventricular hypertrophy as a response to hypoxia-induced pulmonary hypertension in rats (Zhao *et al.*, 2001) and the evidence of its involvement in several cardiopathies in humans (see for example, Tiso *et al.* (2002)). If genomic regions that affect SaO were identified, this information could potentially be used within the commercial breeding programme and contribute to a more rapid improvement of broiler health.

CHAPTER TWO

2 REML ESTIMATION OF GENETIC PARAMETERS FOR BLOOD OXYGEN SATURATION, WEIGHT AT SIX WEEKS AND FLESHING SCORE IN FOUR MEAT-TYPE CHICKEN LINES

2.1 Background and introduction

As reviewed in chapter one, an increase in the incidence of ascites and ascites-related mortality has occurred in broiler populations reared at low altitudes in recent decades. During the last 50 years, improvement in production traits (*e.g.* growth, conformation, feed efficiency) through genetic selection has been spectacular and this has made that the increase in ascites incidence has been linked to this great genetic improvement in production traits. It is now accepted that ascites susceptibility is, at least partially, under genetic control in certain populations. Broiler breeders want to reduce the incidence of the disorder in breeding and commercial flocks and genetic selection against ascites susceptibility provides a more durable means of accomplishing this aim than management solutions, since the benefits are passed from generation to generation. The study of the genetic basis of ascites, and its relationship with growth traits, in meat-type chicken populations reared in commercial conditions is required in order to implement successful breeding programmes that reduce ascites incidence and continue to improve production traits in a sustainable fashion in commercial flocks.

Because ascites presence is only revealed in post-mortem examination (except for severe cases), selection against it has been done using family information. In recent years, a variety of physiological and anatomical measures have been evaluated as indicators of ascites susceptibility. One of these measures is blood oxygen saturation (SaO), which measures oxygen binding to haemoglobin. Julian and Mirsalimi (1992) showed that broilers suffering from ascites had lower SaO than fast or slow growing healthy contemporaries and that fast growing healthy birds had lower SaO than slow growing ones. Wideman *et al.* (1998) demonstrated that low SaO predicted susceptibility to ascites and Druyan *et al.*

(1999) showed that SaO was heritable and that its genetic correlation with ascites mortality was negative in a cold-challenged broiler line. SaO has the advantage of being easily and non-invasively measured in large numbers of birds that can include selection candidates, which makes it convenient to use in commercial breeding programmes.

The aim of this chapter is the estimation of genetic parameters (heritabilities and genetic correlations) for SaO, weight (Weight) and fleshing score (Flesh, a measure of breast conformation) measured at six weeks of age in for four large pedigrees of different meat-type chicken populations.

2.2 Materials and methods

2.2.1 Data description

Data on SaO, weight (in decagrams) and fleshing score (1 to 5 scale, where a high score means a good conformation) were available for four lines of meat-type chicken. Records on SaO were available only for male selection candidates. Other information included hatch week (hatch, with 224 levels for lines 1, 2 and 3 and 113 levels for line 4), sex (sex) and age of dam when the egg was laid (agedam in weeks, with 14 levels).

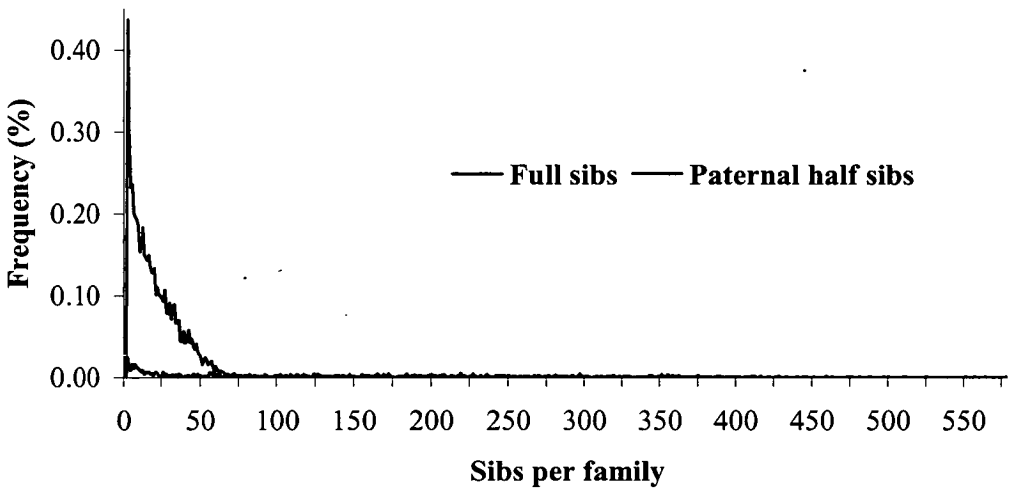
Pedigrees for all lines consisted of around eight generations that were overlapping for all lines except line 4. Table 2.1 shows an overview of pedigree and data structure. We considered "base birds" the first generation of birds available in our pedigrees. These birds were not unrelated or unselected.

Table 2.1. Pedigree and data structure for blood oxygen saturation (SaO), weight (Weight) and fleshing score (Flesh) for the four populations studied.

	Line 1	Line 2	Line 3	Line 4
Total number of birds	179565	320550	134986	206876
Number of base birds	1226	1421	1018	1502
Number of non-base birds	178339	319129	133968	205374
Number of birds with record for SaO	23134	17513	11919	9055
Number of birds with record for Weight and Flesh	133603	318740	118782	205063
Number of sires/paternal half-sib families	935	1174	755	1218
Number of dams	5864	8500	4809	8677
Number of full-sib families	7788	11560	6563	10640

The size of the sibships was very variable within and between lines. Family sizes ranged from one to around 100 individuals per full-sib family (with a mean full-sib family size of 25 birds) and from one to around 580 birds per paternal half-sib family (with a mean half-sib family size of 160 birds). Figure 2.1 shows the distribution of family sizes for line 3 as an example. Distributions for other lines were similar. Not all birds had SaO data (although all had weight and fleshing score records) and the mean number of males with SaO data per recorded full-sib and paternal half-sib family was respectively three and 17.

Figure 2.1. Distribution of full-sib and half-sib family sizes for line 3.



At the time when data were recorded, the overall mortality for these lines was 5-10% and mortality caused by ascites was part of this proportion.

Descriptive statistics of the distributions of SaO, weight and fleshing score were obtained with GENSTAT (GENSTAT 5 COMMITTEE, 1993). In this and the following section, basic results will be presented for all lines but more detailed descriptions will, in some cases, be presented only for line 3, which is the line for which further studies, presented on the next chapters, were carried out.

2.2.2 *Genetic analyses*

Genetic parameters were estimated by Restricted Maximum Likelihood (REML) in a trivariate animal model using ASREML (Gilmour *et al.*, 2000). Fixed effects fitted were hatch and agedam for all three traits and sex for Weight and Flesh (since SaO was only recorded for males). Agedam affects egg characteristics and this can affect progeny traits. For instance, older parents produce larger eggs, which have a greater oxygen demand, but a low surface-area to volume ratio and this is likely to increase ascites incidence in the pre-hatching period. The expression of some traits can be influenced by the ability of the individual's dam to provide a suitable environment. This ability can be partly environmental and partly genetic (see, for example, Mrode (1996)) and in the case of birds reared in commercial conditions, this influence is mostly reduced to the quality and composition of the egg (since, for example, brooding behaviour would be irrelevant in this case). A series of single trait analyses were carried out for all traits. Four different models were considered:

Model 1: including a random animal effect in addition to a residual term.

Model 2: in addition to the terms in Model 1, Model 2 included a maternal environmental component, modelled as a common dam effect for all maternal half-sibs.

Model 3: in addition to the terms in Model 1, Model 3 included a maternal genetic component.

Model 4: including, in addition to the terms in Model 1, both a maternal environmental component and a maternal genetic component.

Stram and Lee (1994) suggested (following Self and Liang (1987)) that, when testing the hypothesis that a given scalar has an unspecified positive value versus the hypothesis that this scalar is zero, the asymptotic distribution of -2 times the logarithm of the likelihood ratio (-2LnLR) is a 50 : 50 mixture of a χ^2 with zero degrees of freedom and a χ^2 with one degree of freedom. The significance of the maternal environmental and genetic components was tested using this criterion and the best model for each trait was chosen for

further (multitrait) analyses. Estimates of variance components from the univariate analyses were used as starting values for trivariate analyses.

2.3 Results

2.3.1 Data description

Table 2.2 presents the means and standard deviations for the raw phenotypic data for the traits analysed. When differences between hatches were accounted for, population means for SaO, Weight and Flesh were not significantly different ($p>0.05$) across populations.

Table 2.2. Means and standard deviations (in brackets) for blood oxygen saturation (SaO), weight (Weight) and fleshing score (Flesh).

	SaO (%)	Weight (dag)	Flesh (units)
Line 1	79.85 (9.38)	255.20 (30.05)	3.19 (0.91)
Line 2	82.32 (8.52)	213.70 (29.26)	3.04 (0.87)
Line 3	80.02 (9.15)	271.60 (33.66)	3.17 (0.92)
Line 4	75.30 (8.95)	226.20 (30.98)	3.09 (0.96)

For all the lines, the distributions of the raw SaO data were non-Normal (negatively skewed, with skewness coefficients ranging from –0.7 to –0.8 and leptokurtic with kurtosis coefficients of around 0.5). Figure 2.2 shows the distribution of SaO phenotypes for all individuals in the pedigree that had an SaO record for line 3 as an example. Distributions for other lines were similar. Figure 2.3 shows mean SaO phenotypes for each hatch plotted over time (hatch number) as well as intervals covering from the first to the third quartile of each hatch distribution. Regressions fitted to means and quartiles show that, over time, mean and first and third quartiles hatch SaO values increased ($p<0.0001$). Fitting different regressions for means and quartiles explained the data better than a model for which only one regression coefficient was fitted ($p<0.005$), and the slope for the first quartile regression was greater than the slope for the mean regression that was in turn greater than the slope for the third quartile regression. This implied that the variance of SaO values decreased over time.

Figure 2.2. Distribution of blood oxygen saturation (SaO) for line 3. Phenotypes of all individuals in the pedigree with SaO record were used to obtain the distribution.

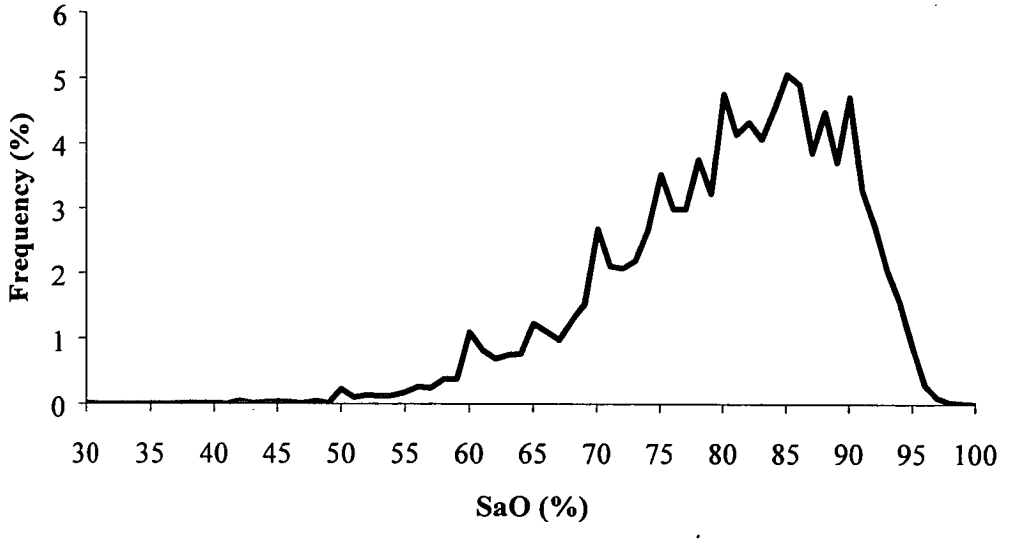
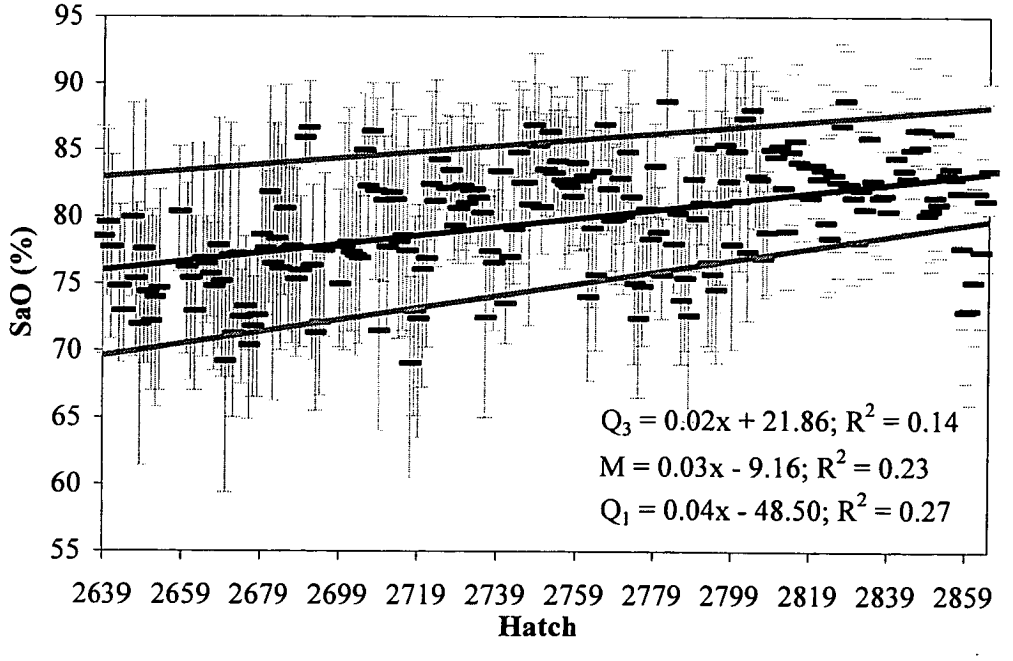
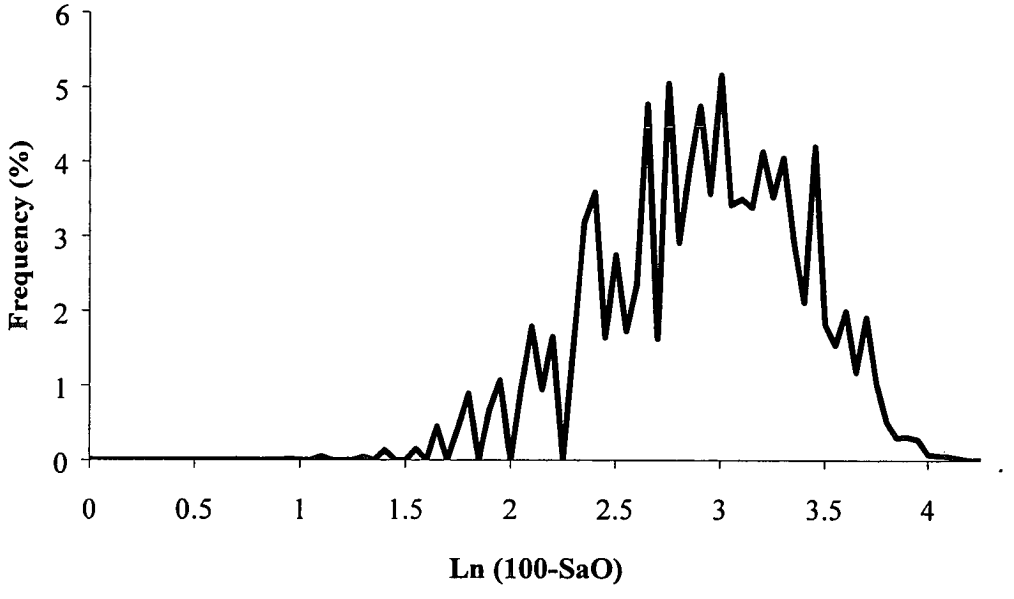


Figure 2.3. Mean (M) hatch SaO phenotypes (-) plotted over time (hatch number) as well intervals covering from the first to the third quartile (Q_1 and Q_3, respectively) of each hatch distribution (in grey). Fitted lines and regressions equations are shown for M (black continuous line), Q_1 and Q_3 (grey continuous lines).



The transformation Ln(100–x), where x is SaO was found to decrease the skewness of the SaO distribution (skewness coefficients from –0.3 to –0.4). Figure 2.4 shows the distribution of the transformed phenotypes for line 3. Genetic parameters were obtained for both untransformed and transformed SaO data.

Figure 2.4. Distribution of Ln(100-SaO) for line 3. Phenotypes of all individuals in the pedigree with SaO record were used to obtain the distribution.



2.3.2 *Genetic analyses*

Throughout this section results are only presented for analyses of untransformed SaO data, since analyses of transformed data yielded similar variance ratios and results obtained on the untransformed scale have an easier interpretation.

Table 2.3 shows the estimates of variance components for untransformed SaO data, weight and fleshing score obtained from univariate analyses for Models 1, 2, 3 and 4. Estimates obtained for the model chosen for further analyses are shown in bold. For some of the analyses of SaO data, the maternal genetic component tended to move past the boundary (zero) and was fixed near it. These analyses are shown in grey and the models that produced

them (within line and trait) were not used in further analyses. Table 2.4 shows -2LnLR obtained from comparison of different models and an indication of the improvement in fit of the full compared to the reduced model (*e.g.* Model 2 compared to Model 1, etc.). In brief, for Weight and Flesh, the "best model" for all lines was Model 4, that included both an environmental and a genetic maternal component. For SaO data, Model 1 was the "best model" for lines 1, 3 and 4, whereas Model 2 (including an environmental maternal component) was the "best model" for line 2.

Table 2.3. REML estimates of genetic, maternal environmental and residual variances obtained from univariate analyses for blood oxygen saturation (SaO), body weight (Weight) and fleshing score (Flesh) for all lines studied.

		Model 1			Model 2				Model 3				Model 4				
		σ_u^2	σ_e^2	σ_p^2	σ_u^2	σ_{em}^2	σ_e^2	σ_p^2	σ_u^2	σ_{gm}^2	σ_e^2	σ_p^2	σ_u^2	σ_{em}^2	σ_{gm}^2	σ_e^2	σ_p^2
Line 1	**SaO**	**11.29**	**58.00**	**69.28**	10.84	0.23	58.11	69.19	10.72	0.25	58.22	69.19	10.60	0.11	0.21	58.24	69.16
	Weight	239.50	277.04	516.54	171.76	12.48	308.21	492.45	192.46	13.71	298.45	504.63	**168.45**	**9.87**	**4.29**	**309.88**	**492.48**
	Flesh	0.24	0.62	0.86	0.18	0.01	0.65	0.84	0.21	0.01	0.64	0.85	**0.18**	**0.01**	**0.00**	**0.65**	**0.84**
Line 2	**SaO**	9.69	54.13	63.82	**7.66**	**1.09**	**54.66**	**63.42**	9.47	0.11	54.22	63.79	7.67	1.09	0.00	54.65	63.41
	Weight	176.29	173.76	350.05	116.55	9.27	201.95	327.77	130.77	11.59	195.07	337.42	**113.59**	**6.23**	**4.66**	**203.41**	**327.88**
	Flesh	0.26	0.55	0.81	0.18	0.01	0.59	0.78	0.21	0.01	0.58	0.80	**0.18**	**0.01**	**0.00**	**0.59**	**0.78**
Line 3	**SaO**	**14.13**	**54.17**	**68.29**	12.07	1.20	54.60	67.88	13.48	0.37	54.37	68.21	12.07	1.21	0.00	54.61	67.88
	Weight	271.74	382.80	654.54	164.81	17.87	433.54	616.22	195.25	19.65	419.00	633.90	**161.86**	**13.49**	**6.71**	**434.90**	**616.96**
	Flesh	0.27	0.62	0.89	0.19	0.02	0.66	0.87	0.22	0.01	0.64	0.88	**0.19**	**0.01**	**0.00**	**0.66**	**0.86**
Line 4	**SaO**	**8.02**	**57.32**	**65.35**	7.98	0.04	57.32	65.34	8.15	0.00	57.23	65.38	8.20	0.01	0.00	57.17	65.38
	Weight	234.07	214.14	448.21	183.21	28.29	231.01	442.50	185.74	48.48	229.06	463.27	**181.22**	**9.42**	**32.46**	**231.21**	**454.30**
	Flesh	0.47	0.52	0.99	0.41	0.01	0.55	0.97	0.42	0.02	0.54	0.98	**0.40**	**0.01**	**0.01**	**0.55**	**0.97**

Table 2.4. -2 times the logarithm of the likelihood ratio (-2LnLR) obtained for comparison of models used in univariate analyses for blood oxygen saturation (SaO), body weight (Weight) and fleshing score (Flesh) for all lines studied.

		Model 1 vs. Model 2		Model 1 vs. Model 3		Model 2 vs. Model 4		Model 3 vs. Model 4	
		-2LnLR	p-value	-2LnLR	p-value	-2LnLR	p-value	-2LnLR	p-value
Line 1	SaO	0.40	0.26	0.80	0.19	NA	NA	NA	NA
	Weight	188.00	<0.001	142.00	<0.001	16.00	<0.001	62.00	<0.001
	Flesh	89.80	<0.001	56.40	<0.001	8.80	<0.005	42.20	<0.001
Line 2	SaO	7.80	<0.005	0.20	0.33	NA	NA	NA	NA
	Weight	500.00	<0.001	520.00	<0.001	120.00	<0.001	100.00	<0.001
	Flesh	222.00	<0.001	160.00	<0.001	30.00	<0.001	92.00	<0.001
Line 3	SaO	2.10	0.07	0.80	0.19	NA	NA	NA	NA
	Weight	162.00	<0.001	132.00	<0.001	18.00	<0.001	48.00	<0.001
	Flesh	82.20	<0.001	49.00	<0.001	5.20	0.01	38.40	<0.001
Line 4	SaO	0.00	0.50	NA	NA	NA	NA	NA	NA
	Weight	1428.00	<0.001	1694.00	<0.001	302.00	<0.001	36.00	<0.001
	Flesh	131.20	<0.001	154.00	<0.001	47.40	<0.001	24.60	<0.001

NA: not available.

Although the "best model" for Weight and Flesh for all lines was Model 4, trivariate analyses including a maternal genetic component could not be run for lines 2 and 4 due to the large size of these pedigrees. Instead results are presented for (trivariate) analyses not including a genetic maternal component but including an environmental maternal component for all traits (line 2) or for Weight and Flesh (line 4).

Table 2.5 shows heritabilities (h^2, on the diagonal), genetic (r_g) and phenotypic (r_p) correlations and their standard errors, obtained for (untransformed) SaO, Weight and Flesh. Variance ratios and correlations are also shown for the environmental (c^2) and genetic (g^2) maternal components when available. h^2 for SaO varied in the range [0.12; 0.21], the lowest value corresponding to lines 2 and 4 and the highest to line 3. In univariate analyses, only line 2 showed significant environmental maternal effects for SaO, and these explained around 2% of the total variance. The significance of the maternal environmental component for SaO was retested in trivariate analyses. The full model included one variance (c^2 for SaO) and two covariances more than the reduced model and -2LnLR was therefore compared to a 50 : 50 mixture of a χ^2 with two degrees of freedom and a χ^2 with three degrees of freedom (Self and Liang, 1987). The results obtained from these tests were consistent with univariate results.

h^2 for Weight ranged between 0.26 and 0.41, the lowest value corresponding to line 3 and the highest to line 4. The inclusion of both environmental and genetic maternal effects improved significantly the fit of the model in univariate analyses, and these components explained between 2 and 6% and 1% respectively (g^2 was only available for lines 1 and 3) of the total variance. Line 4 was the one for which the environmental maternal component explained the largest proportion of variance compared to other lines.

h^2 for Flesh was approximately in the same range than h^2 for Weight and its lowest value (0.22) corresponded to lines 1 and 3, the highest (0.41) corresponding to line 4.

Environmental and genetic maternal effects jointly explained under 3% of the total variance for this trait.

Genetic correlations of SaO with Weight were low and negative ([-0.17;-0.02]) for all lines and were not significantly ($p>0.05$) different from zero for line 3. Genetic correlations of SaO with Flesh were approximately in the same range and were not significantly ($p>0.05$) different from zero for lines 2 and 3. Genetic correlations between the two production traits were positive and moderate to high (0.31 for line 4 and 0.64 for line 2). Correlations of environmental and genetic maternal effects were generally high between these two traits and approached unity in some cases.

Table 2.6 shows the estimates of genetic, maternal environmental, maternal genetic, and residual and phenotypic variances (σ_u^2, σ_{em}^2, σ_{gm}^2, σ_e^2 and σ_p^2, respectively) obtained from trivariate analyses for SaO, Weight and Flesh for all lines studied. The fact that the estimated h^2 of SaO for line 3 was almost twice the value estimated for lines 2 and 4 reflects differences in estimated σ_u^2 rather than differences in σ_e^2. σ_e^2 for Weight appeared to be greater for line 3 than for other lines and this was reflected in a lower estimated h^2 for this line. The estimated σ_u^2 for Flesh was almost double for line 4 than for the other lines, and h^2 of Flesh for this line was twice the value estimated for the others.

The heritabilities obtained when performing the analysis using transformed SaO phenotypes did not differ from the ones obtained from untransformed data and are therefore not presented. Because of the nature of the transformation, genetic and phenotypic correlations with Weight and Flesh were different in sign but not different in magnitude.

Table 2.5. Heritabilities (on diagonals and bold at "Direct Genetic and Phenotypic" column), genetic and phenotypic correlations (below and above diagonal respectively) and their standard errors (in brackets), obtained for blood oxygen saturation (SaO), weight (Weight) and fleshing score (Flesh). Variance ratios and correlations are also presented for the environmental and genetic maternal components when relevant.

		Direct Genetic and Phenotypic			Maternal Environmental			Maternal Genetic		
		SaO	Weight	Flesh	SaO	Weight	Flesh	SaO	Weight	Flesh
Line 1	SaO	**0.17 (0.01)**	-0.00 (0.01)	-0.04 (0.01)						
	Weight	-0.17 (0.04)	**0.34 (0.01)**	0.57 (0.00)		0.02 (0.00)			0.01 (0.00)	
	Flesh	-0.21 (0.04)	0.59 (0.02)	**0.22 (0.01)**		0.76 (0.05)	0.01 (0.00)		0.87 (0.12)	0.00 (0.00)
Line 2	SaO	**0.12 (0.02)**	-0.02 (0.01)	-0.07 (0.01)	0.02 (0.00)					
	Weight	-0.13 (0.06)	**0.35 (0.01)**	0.58 (0.00)	0.00 (0.12)	0.03 (0.00)				
	Flesh	-0.11 (0.06)	0.64 (0.01)	**0.24 (0.01)**	-0.07 (0.13)	0.90 (0.02)	0.02 (0.00)			
Line 3	SaO	**0.21 (0.02)**	0.01 (0.01)	-0.04 (0.01)						
	Weight	-0.02 (0.06)	**0.26 (0.01)**	0.57 (0.00)		0.02 (0.00)			0.01 (0.00)	
	Flesh	-0.10 (0.06)	0.53 (0.03)	**0.22 (0.01)**		0.68 (0.00)	0.01 (0.00)		0.77 (0.00)	0.00 (0.00)
Line 4	SaO	**0.12 (0.02)**	-0.02 (0.01)	-0.05 (0.01)						
	Weight	-0.17 (0.07)	**0.41 (0.01)**	0.38 (0.00)		0.06 (0.00)				
	Flesh	-0.15 (0.06)	0.31 (0.02)	**0.41 (0.01)**		0.62 (0.00)	0.02 (0.00)			

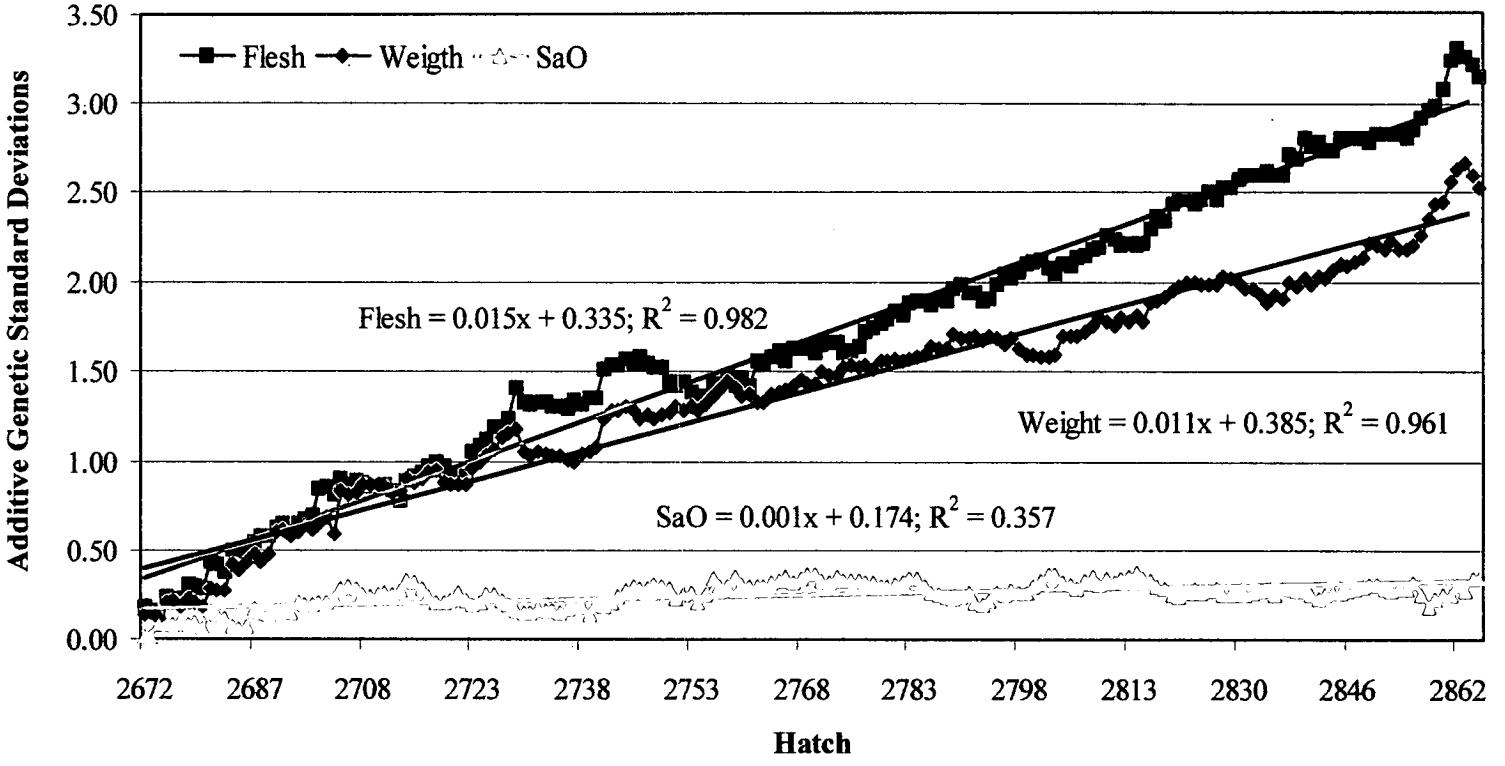
Table 2.6. REML estimates of genetic, maternal environmental, maternal genetic, residual and phenotypic variances obtained for blood oxygen saturation (SaO), weight (Weight) and fleshing score (Flesh) for all lines studied.

		σ_u^2	σ_{em}^2	σ_{gm}^2	σ_e^2	σ_p^2
Line 1	**SaO**	11.53	NA	NA	57.88	69.41
	Weight	165.90	10.27	4.08	311.10	491.35
	Flesh	0.19	0.01	0.00	0.64	0.85
Line 2	**SaO**	7.97	1.09	NA	54.61	63.67
	Weight	114.50	9.47	NA	203.00	326.97
	Flesh	0.19	0.01	NA	0.58	0.79
Line 3	**SaO**	14.37	NA	NA	54.06	68.43
	Weight	158.80	13.76	6.80	436.40	615.76
	Flesh	0.19	0.01	0.00	0.66	0.87
Line 4	**SaO**	8.09	NA	NA	57.34	65.43
	Weight	180.50	28.84	NA	232.31	441.65
	Flesh	0.40	0.01	NA	0.55	0.96

NA: not available.

Figure 2.5 shows the mean additive value (in additive standard deviations) for SaO, Weight and Flesh for each hatch for line 3. Estimated breeding values from all birds in the pedigree (not only the ones with records for SaO) were used to obtain the graph. The figure shows that, although mean hatch values increased over time for all three traits, the rate of increase was a greater for Flesh and Weight than for SaO.

Figure 2.5. Mean additive value (in additive standard deviations) for SaO, Weight and Flesh for each hatch for line 3. Regression equations of fitted lines are also shown.



2.4 Discussion

We have demonstrated that there was substantial genetic variation (h^2 of approximately 0.1- 0.2) for SaO in the four populations studied and therefore genetic selection on this trait can be carried out. Although a slightly higher heritability and additive genetic variance was obtained for SaO in line 3, in general the estimates of variances, heritabilities and genetic correlations with weight and fleshing score are consistent across lines. Druyan *et al.* (1999) obtained estimates of h^2 for SaO of around 0.5-0.6. Unlike our populations, the population Druyan *et al.* (1999) used in their study was cold-challenged, and this is common in studies of ascites-related traits (see Table 2.7), with few exceptions (*e.g.* Moghadam *et al.* (2001)). Rearing birds in challenging environments increases the incidence of the disorder and therefore facilitates selection against it. Table 2.7 shows heritabilities of a selection of ascites-related traits and genetic correlations with body weight from available studies. All traits selected are heritable, but estimates of heritabilities varied widely across studies for similar traits. Moghadam *et al.* (2001) estimated that h^2 for ascites mortality obtained from male data alone were higher than the ones obtained jointly from male and female data.

The heritabilities obtained for body weight are similar to the ones reported in the literature (0.2-0.4) (*e.g.* Dunnington and Siegel (1996), Koerhuis and Thompson (1997)). Despite decades of selection on growth or body weight, genetic variation has not been depleted for these (Rose, 1997) or related traits such as feed conversion (Emmerson, 1997); heritabilities are still moderate to high in contemporary broiler strains and have not diminished markedly compared with earlier estimates (Hill, 1996). The estimates of h^2 obtained for fleshing score were of the same size of those for body weight, and although selection on this trait has not been taking place for as long as for growth traits, the maintenance of this level of genetic variation is also remarkable.

Table 2.7. Selection of ascites-related traits and estimates of heritabilities (h^2) and genetic correlations with body weight (r_g (Weight)).

Trait	h^2	r_g (Weight)	Cold Challenged?	Comments	Reference
Ascites mortality	0.11-0.44	NA	Yes		Lubritz *et al.* (1995)
	0.06	NA	Yes		De Greef *et al.* (2001a)
	0.12-0.21	NA	No	males and females	Moghadam *et al.* (2001)
	0.22-0.41	0.22-0.35	No	males	Moghadam *et al.* (2001)
Total mortality	0.22	-0.46	Yes		De Greef *et al.* (2001a)
	0.16	NA	Yes	maternal genetic effects significant	Pakdel *et al.* (2002a)
	0.32	-0.06	Yes	maternal genetic effects not considered	Pakdel *et al.* (2002b)
Heart-API	0.21-0.27	NA	Yes		Lubritz *et al.* (1995)
	0.54	-0.26	Yes		De Greef *et al.* (2001a)
	0.28	NA	Yes	maternal genetic effects significant	Pakdel *et al.* (2002a)
	0.45	-0.27	Yes	maternal genetic effects not considered	Pakdel *et al.* (2002b)
	0.42-0.72	NA	Yes		Druyan *et al.* (1999)
PCV	0.50	-0.54	Yes		De Greef *et al.* (2001a)
	0.50	NA	Yes		Pakdel *et al.* (2002a)
	0.46	-0.23	Yes	maternal genetic effects not considered	Pakdel *et al.* (2002b)
	0.74-0.88	NA	Yes		Druyan *et al.* (1999)
SaO	0.53-0.63	NA	Yes		Druyan *et al.* (1999)
APO_2	0.13	0.12	Yes		De Greef *et al.* (2001a)
Troponin T	0.38	NA	NA		Maxwell *et al.* (1998)
Heart rate	0.52-0.69	NA	Yes		Druyan *et al.* (1999)

Heart-API: ratio of the weight of the right heart ventricle to total heart weight.
PCV: packed cell volume or heamatocrit.
APO_2: "arterial oxygen pressure" (directly related with SaO).

Hill (1996) suggested that mutational variance could be an important source of new genetic variation. Also, the fact that there is still genetic variation may support the idea that many genes with very small effects influence those characters. Alternatively, the mode of action of individual genes, directly on these production traits or indirectly through actions on fitness traits, and interactions amongst loci could explain why genetic variation is maintained; Hill (2002) also pointed out that, under certain circumstances, selection favoured most variable genotypes. Breeding strategies also influence changes of genetic variance over time: for example, new variation could be "brought in" by the incorporation to the population of "immigrants" from different lines and fixation of unfavourable alleles could be prevented in this way; this influence could as well be through the selection strategy implemented, for instance the use of mass selection during the early years of the poultry breeding industry may have contributed to maintaining high effective population sizes (Hill, 1996).

Pakdel *et al.* (2002a) and Koerhuis and Thompson (1997) demonstrated, respectively, the significance of genetic maternal effects on ascites related traits and environmental and genetic maternal effects on body weight. In our study, the inclusion of a maternal environmental component for SaO only improved significantly the fit of the model for line 2. The structure of our SaO data may have had an impact on our results: a large proportion (around 30%) of maternal half-sib families were of size one, and the mean maternal family size for SaO data was around three. Datasets with larger maternal family sizes could facilitate a better estimation of environmental maternal effects for SaO and allow exploration of the existence of genetic maternal effects for this trait in our populations. Significance of the maternal environmental component was re-tested in trivariate analyses yielding similar results to the ones obtained in univariate analyses. In the case of Pakdel *et al.* (2002a), although the genetic maternal component (g^2) explained under 5% of the total variance for all traits, its inclusion in the analysis model had a large impact on the estimated heritability, halving it for most traits, and the correlation of the additive and the maternal

genetic components was moderate or high depending on the traits. In our case, although the inclusion of a maternal environmental component slightly decreased heritability for line 2, this decrease was only small, as was the decrease in estimated additive genetic variance. Maternal family sizes for Weight and Flesh data were considerably greater than for SaO data, allowing for the estimation of both environmental and genetic maternal effects.

Koerhuis and Thompson (1997) estimated that environmental and genetic maternal effects explained respectively fewer than 10% and 5% of the total variance for juvenile body weight. In their case, environmental maternal effects were divided into both common maternal half-sib effects and common full-sib family effects. Our results show that both heritable and non-heritable maternal effects are significant for both weight and fleshing score, although the percentage of the total variance explained jointly by these effects was under 10% for both traits across lines. Their inclusion in the analysis model reduced estimated heritabilities generally more markedly for weight than for fleshing score. We attempted to estimate the correlation between the direct additive (*i.e.*, animal) component and the genetic maternal component, but in the cases where the size of the pedigree allowed the analyses to be run, those failed to converge and therefore no results have been presented. For lines 2 and 4, multitrait analyses including a genetic maternal component could not be run and results were presented instead for analyses including only an environmental genetic component. This, most likely implies that σ_{em}^2 would be overestimated and would include part of σ_{gm}^2. Based on results from univariate analyses, genetic maternal effects would be larger than environmental ones for line 4 but approximately of the same size for line 2.

Since ascites is a production-related disorder caused by high oxygen requirements of rapid growth, one would expect a positive correlation between growth-related traits and incidence of the disorder. Since SaO is negatively correlated with ascites susceptibility, the sign of our estimates of genetic correlations between SaO and Weight and Flesh meets the expectation. Nevertheless, the estimated correlations of SaO with production traits were relatively low, suggesting that selection to increase SaO (and therefore reduce ascites

susceptibility) and production traits simultaneously would be possible in the populations studied. Correlations of other ascites indicator traits with body weight are shown in Table 2.7. These correlations are not always consistent with expectations. For instance, De Greef *et al.* (2001a) estimated negative genetic correlations between body weight and total mortality and heart-API (amongst others) and these traits are highly positively correlated with ascites mortality or presence. The authors argued that the sign of their estimated correlations was not as expected because genetic correlation estimates are affected by the presence of the disorder and that this effect depends on the proportion of affected individuals. They suggested that this phenomenon can be viewed as a genotype x environment interaction, in which the "internal environment" of the bird (ascites presence or absence) might affect the ability of fast growing animals to fully express their growth potential, so they would show below-average growth. At the same time, changes in metabolic pressure caused by increased growth trigger the presence of the disorder in genetically prone birds that would not be affected if growth were somehow controlled by management practices and growth acts as an "internal environment" that affects ascites presence. These ideas were previously suggested by Julian (1993). To check their hypothesis, they analysed a subset of data containing only animals not affected with ascites (the dataset was divided on the basis of heart-API observed values). The sign of the correlation obtained from this data set changed, meeting their original expectations. Since our populations were not cold-challenged, presumably the incidence of the disorder was lower than for the population studied by De Greef *et al.* (2001a) and the effect on estimated genetic correlations would be smaller. To check the effect that estimating genetic correlations from subsets of potentially ascitic (*i.e.*, with low SaO records) and non-ascitic birds had in our case we focused on line 3 and divided the dataset into "ascitic" (with SaO records lower than 60 or 75) and "non-ascitic" birds (with SaO records higher than 60, 80, 90 or 95). Table 2.8 shows estimates of heritabilities and genetic correlations for the traits studied.

Table 2.8. Estimates of heritabilities (h^2) and genetic correlations (r_g) for SaO, Weight and Flesh obtained from different subsets of line 3 data set. The original data set was divided on the basis of SaO measures, in an attempt to separate ascites-susceptible from ascites-resistant birds. N is the number of birds with records per subset of data.

	Cut-off SaO	N	r_g (SaO,Weight)	r_g (SaO,Flesh)	r_g (Weight,Flesh)	h^2 (SaO)	h^2 (Weight)	h^2 (Flesh)
Ascites susceptible	< 60	315	0.30 (0.36)	0.37 (0.38)	0.48 (0.03)	0.19 (0.29)	0.23 (0.01)	0.18 (0.01)
	< 75	3062	-0.23 (0.22)	-0.09 (0.21)	0.48 (0.03)	0.03 (0.02)	0.23 (0.01)	0.19 (0.01)
Full dataset	None	11919	-0.02 (0.06)	-0.10 (0.06)	0.53 (0.03)	0.21 (0.02)	0.26 (0.01)	0.22 (0.01)
Ascites resistant	> 60	11506	-0.04 (0.06)	-0.12 (0.06)	0.54 (0.03)	0.16 (0.02)	0.26 (0.01)	0.22 (0.01)
	> 80	6499	0.16 (0.13)	-0.02 (0.12)	0.50 (0.03)	0.04 (0.01)	0.24 (0.01)	0.20 (0.01)
	> 85	3871	0.08 (0.17)	0.10 (0.16)	0.48 (0.03)	0.04 (0.02)	0.24 (0.01)	0.19 (0.01)
	> 90	1299	0.29 (0.32)	0.07 (0.28)	0.47 (0.03)	0.04 (0.05)	0.23 (0.01)	0.18 (0.01)

Although estimated heritabilities of production traits and genetic correlations amongst them were similar for all subsets, this was not the case for SaO and correlations of this trait with Flesh and Weight (N.B. these correlations were not different from zero ($p>0.05$) in any case, possibly due to the small sample size of some subsets). Estimated heritabilities for SaO varied across subsets, and were close to zero for subsets of birds with SaO records smaller than 75% or greater than 80% SaO. For these subsets, estimated additive genetic variances were smaller than or equal to one. The estimated phenotypic variance for the subsets with SaO records smaller than 75% was around 35. The estimated phenotypic variance for subsets with SaO records greater than 80% varied from 13 to 2 and presumably few susceptible or affected animals were present in these subsets. In these cases, the expected sign of the correlation with production traits would be negative (since the expected sign of the genetic correlation between production and ascites incidence would be positive and SaO is negatively correlated with ascites incidence), and this was not observed. For subsets of birds with less than 60% SaO and more than 60% SaO, heritabilities for this trait were similar despite differences in total phenotypic variance (respectively, 22 and 52 for the two sets of data). The estimated genetic correlations of SaO with production traits obtained from the subset of birds with less than 60% SaO were positive (as expected for affected birds) and moderate (apparently larger than the one estimated from the whole dataset, but caution must be taken in the interpretation of this result given the large standard error of the estimate). In conclusion, the proportion of susceptible birds (assigned on the basis of SaO records) seems to have an effect on the estimated genetic correlations between SaO and production traits, but no clear trends were identified, and differences in sample size of the different subsets make results still more difficult to interpret. These analyses are also difficult to interpret in that the estimation of parameters was carried out for the trait used to "censor" the datasets. Anyhow, if these effects were real, they would have an impact on the success of programs aiming to improve SaO and production traits simultaneously.

McMillan and Quinton (2002) conducted a simulation study to assess the changes in ascites incidence when selection was performed on body weight alone or also on an ascites indicator trait, under a variety of scenarios. They concluded that selection for improved growth and reduced ascites could be effective, more so if an indicator trait was used together with sib information prior to selection for growth, but noticed that this reduced gain in the latter trait. The authors showed that the higher the correlation of the indicator trait with ascites, the greater was the genetic reduction of the population's ascites susceptibility. We do not have estimates of correlations between SaO and ascites incidence in our populations, but Druyan *et al.* (1999) estimated that the genetic correlation of SaO with ascites mortality was around –0.50. McMillan and Quinton (2002) also observed that a reduction in the genotypic mean of ascites susceptibility did not always translate into a decrease in the incidence of the disorder, but rather the opposite, and suggested that this was an effect of added metabolic pressure of the improvement in growth potential and therefore a change in the "internal environment" of the birds. However, this result could depend on the model used in their simulations.

Finally, Fig. 2.5 shows that, although heritabilities for SaO, Weight and Flesh were similar for line 3, and genetic progress was being done on the three traits studied improvement was more pronounced for production traits. This could be a consequence of breeding strategies but could also be caused by a failure of the models used to adequately describe the genetic architecture of the traits studied and their genetic relationships. In further chapters more complex models to describe the genetic architecture of SaO are studied and its relationship with weight and fleshing score is explored in more depth. Particularly, we explore the possibility that a locus or several loci with large effect control blood SaO levels and therefore potentially ascites susceptibility, since identification of such loci could make the reduction of ascites incidence in broiler flocks an easier task, allowing for direct manipulation of allele frequencies at this locus or loci, therefore bypassing potential

problems due to effects of allele, gene or genotype x environment interactions that could hinder the effectiveness of more traditional selection methods.

CHAPTER THREE

3 SEGREGATION ANALYSIS OF BLOOD OXYGEN SATURATION DATA

3.1 Introduction

We have demonstrated in the previous chapter the existence of substantial genetic variation for blood oxygen saturation (SaO) for a set of four meat type chicken lines. Estimates of heritabilities for this trait ranged from 0.1 to 0.2. These estimates were obtained assuming an infinitesimal model (Fisher, 1918), *i.e.*, we assumed that the (quantitative) trait was influenced by an infinite number of unlinked loci (polygenes), each with an infinitely small additive effect on the trait. However, in recent years, several studies have shown that one or few genes, or quantitative trait loci (QTL), explain an important amount of the phenotypic or genetic variation for some quantitative traits. Today, examples of genes or QTL with large effect on quantitative traits of agricultural interest are numerous. Among them are the halothane sensitivity gene (Jensen and Barton-Gade, 1985) and the RN- mutation in pigs (Milan *et al.*, 2000) that affect meat quality and meat content, respectively, the Booroola (Piper and Bindon, 1992) and the callipyge gene (Cockett *et al.*, 1994) in sheep that affect ovulation rate and leanness and feed efficiency, the double muscling gene (Hanset and Michaux, 1985a; Hanset and Michaux, 1985b) and the DGAT1 gene (Grisart *et al.*, 2002) in cattle, that affects milk fat content and other milk characteristics and the dwarfing gene in poultry (Merat and Ricard, 1974), that has an effect on growth and fatness.

Little is known about the genetics underlying ascites-related traits. In this chapter, we study the possible existence of a major gene or quantitative trait locus (QTL) involved in the control of SaO. To this end, we have analysed the data from one of the lines previously studied using a mixed inheritance model that includes a major locus as well as polygenes. In subsequent analyses, we have used the estimated genotype probabilities at the putative major locus to investigate its effect on body weight and fleshing score.

Mixed inheritance models were first introduced in human genetics to discriminate between modes of inheritance (Elston and Stewart, 1971; Morton and Maclean, 1974) and

they were used within a maximum likelihood framework, which restricted their practical use to the analysis of small pedigrees. Although a series of approximations of the mixed inheritance model likelihood are available for animal breeding populations with simple structures (see, for example, Knott *et al.* (1992a)), analysis of large complex pedigrees (like the ones usually encountered in animal breeding) is only feasible when sampling based techniques are used either to estimate likelihoods (Guo and Thompson, 1991; Guo and Thompson, 1994) or to implement Bayesian analysis (Janss *et al.*, 1995).

Markov Chain Monte Carlo (MCMC) sampling methods provide an efficient means to carry out these tasks. In particular the Gibbs Sampler, an MCMC method, is now widely used in genetic analyses. It is capable of generating samples from the joint distribution (usually complex) of several random variables by sampling from known and simple conditional distributions. From these samples, marginal distributions of each variable can be obtained and used in Bayesian inference (they are estimates of the posterior distributions of the model parameters).

3.2 Materials and methods

3.2.1 *Data*

In a previous chapter, we estimated heritabilities of blood oxygen saturation (SaO), body weight (Weight) and fleshing score (Flesh) and genetic correlations between these traits for four meat-type chicken lines. Here, we investigate the genetic architecture of SaO in line 3, for which we obtained the highest estimates of genetic variance and heritability for SaO. Furthermore, this line shows a slightly higher ascites-related mortality than other Aviagen Ltd. lines (A. Koerhuis, personal communication) and it is the heaviest of the lines studied.

Several data sets were available from this population:

A. *Data set 1* is the same data set used in the previous chapter to estimate genetic parameters. Records for all traits were taken at six weeks of age and SaO data were only available for male selection candidates.

B. *Data set 2* consisted of data from the same line but collection of data for this set started as data collection for set 1 ended. This break occurred because although the same traits were recorded, the age of measurement changed from six weeks in set 1 to five weeks in set 2.

C. *Data set 3* is the same as data set 2 plus an extra generation of phenotypic information not only on male selection candidates but also including their sibs and other contemporary birds (both male and female). The age of measurement was five weeks.

3.2.2 Statistical analyses

A different set of analyses was carried out for each data set. Details on the analyses are given below but, in brief, we used data set 1 to assess the possibility that a locus with large effect was involved in the genetic control of SaO. Then, data set 2 was analysed to confirm or refute the results obtained with data set 1. Since results from more recent data are of greatest interest if follow up studies were to be carried out or our findings were to have an impact on breeding programme decisions, a more comprehensive set of analyses was done on this data set. Finally, data set 3 was analysed to study the impact of adding more phenotypic information from later generations.

3.2.2.1 Segregation analysis

3.2.2.1.1 Statistical model

A mixed inheritance model was used for the segregation analysis. Mixed inheritance models are a combination of infinitesimal and finite gene models. In our analyses, a single major locus was modelled in addition to a polygenic effect. The major locus was assumed to be autosomal and biallelic with Mendelian transmission probabilities and with an additive (a) and a dominance effect (d). The genotypic value for birds with genotype BB at the major locus is a, $-a$ for bb birds and d for Bb birds. The major locus was assumed to be in Hardy-

Weinberg equilibrium proportions in the "base generation" (*i.e.*, the first generation of a data set). The fixed effects considered in the analyses varied and are described in each section.

The mixed model equation that describes the model fitted to the data is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{ZWm} + \mathbf{e} \qquad [1]$$

where **y** is the vector of phenotypic observations, **b** is the vector of fixed non-genetic effects and **X** is the design matrix relating fixed non-genetic effects to observations. **Z** is the incidence matrix for random polygenic effects ($\mathbf{u} \sim N(0, A\sigma_u^2)$, where **A** is the numerator relationship matrix and σ_u^2 is the polygenic variance) and single locus effects. **W** is a three column matrix that contains information on the genotype of each individual and **m** is the vector of major-genotype means ($\mathbf{m}' = [\ -a,\ d,\ a\ ]$), hence **Wm** is the vector of random effects at the single locus. **e** ($\sim N(0, I\sigma_e^2)$) is a vector of random errors.

3.2.2.1.2 Gibbs sampling and Bayesian analysis

Janss *et al.* (1995) proposed an efficient sampling scheme to use the Gibbs sampler for the study of mixed inheritance models in animal populations. In our analyses, carried out with software developed at Roslin Institute by Ricardo Pong Wong, we used the sampling scheme they described (see Janss *et al.* (1995) and Janss *et al.* (1997a) for details) to obtain marginal posterior distributions for the major locus parameters (frequency and additive and dominance effect), population mean and polygenic and residual variances. For each iteration of the Gibbs sampler, every bird was assigned a genotype. It is possible to have an estimate, averaging over all iterations, of the major locus genotype probabilities (p_{BB}, p_{Bb} and p_{bb}) for each bird in the pedigree.

The variance explained by the major locus (σ_m^2) is defined as:

$$\sigma_m^2 = 2\,p_B\,(1 - p_B)\,[a + d\,((1 - p_B) - p_B)]^2 + [2\,p_B\,(1 - p_B)\,d]^2 \qquad [2]$$

(Falconer and Mackay, 1996) and was computed from the major locus genotypic effects and allele frequency sampled at each iteration. Likewise, we calculated the degree of dominance as d/a.

We used non-informative prior distributions, uniform on (-∞ ; +∞) for fixed non-genetic effects and *d*, on [0 ; +∞) for *a* and on [0 ; 1] for major allele *B* frequency. We used an inverse-gamma prior distribution on (0 ; +∞) for variances with a flat prior for log(variance). This type of prior distribution for variances should cause the mean of the marginal posterior distributions to tend towards zero if the data available do not support variation of the random effects. All genotypes were initialised as *Bb*.

Multiple runs of the Gibbs sampler were carried out for each analysis, with different starting values. Differences in parameter estimates obtained from different chains may reflect problems of mixing.

The number of chains and burn-in period used for each analysis are detailed in the relevant sub-sections.

Marginal posterior distributions of the sampled parameters obtained from each run of the Gibbs sampler were studied. As an example, a graphical summary of one of the chains is presented for the major locus additive effect. For selected analyses, the marginal posterior means, that is the parameter's *a-posteriori* expectation, and standard deviations are reported. The Monte Carlo standard deviation of the marginal posterior mean was computed following Geyer (1992) as suggested by Sorensen *et al.* (1995) and the effective number of samples per chain, *i.e.* the number of independent samples per chain for each parameter, was estimated. Following the results obtained when studying the behaviour of the individual chains for data set 1, no formal assessment of convergence was carried out for data set 2 and 3 analyses. Nonetheless, a visual inspection of individual chains was carried out for each of the analyses. After studying individual chains, the samples were pooled across chains and the mean of the pooled distribution and its standard deviation were used as a summary statistics. Janss *et al.* (1995) suggested the use of the ratio of the density at $\sigma_m^2 = 0$ of the marginal posterior distribution of σ_m^2 and the density at the global mode as a criterion to test the significance of the single locus component. They inferred the presence of a single locus (0.05 significance

level) if the density at the global mode was 20 times larger than that at $\sigma_m^2 = 0$. We used the same criterion.

3.2.2.1.3 Details of analyses for each data set

Data set 1: In order to ease computation, the complex segregation analysis of data set 1 was carried out using SaO phenotypes adjusted for the fixed effects of hatch week (210 levels) and age of the dam at the age of laying (10 levels). Adjusted SaO phenotypes (ADJ) were obtained from the analysis of the SaO, Weight and Flesh data fitting a trivariate animal model using ASREML (Gilmour *et al.*, 2000). This analysis assumed that all three traits are under the genetic control of an infinite number of loci with small additive effects. If a major locus was involved in the genetic control of a trait, its segregation variance would contribute to the estimated σ_u^2 with the remainder included in the estimated σ_e^2, together with polygenic non-additive variance, since the infinitesimal model does not accommodate non-additive genetic variation (although it can be extended to do so) or changes in variance caused by changes in major locus allele frequency (Turelli and Barton, 1994). Only adjusted phenotypes from birds that originally had SaO records were used, but the pedigree included contemporary unrecorded birds. This allowed us to obtain genotype probabilities for all birds in the pedigree. We ran six chains in total for the analysis of data set 1, with different starting values. Individual chains were composed by 255000 iterations that were collected after allowing for a burn-in period of 5000 iterations, keeping each 100^{th} iteration from this point onwards. In order to assess if the burn-in period and thinning parameter we used were adequate, we studied the convergence and parameter estimates of each chain.

Data set 2: First, we carried out the segregation analysis on the adjusted phenotypes as described for data set 1, including in the pedigree contemporary unrecorded birds. The results from this analysis can directly be compared to the results obtained from data set 1. For this analysis, we ran, as previously, six chains of 255000 iterations, keeping each 100^{th} iteration after a burn-in period of 5000. For subsequent analysis, we had access to more efficient computer facilities. Since the effective number of samples per chain obtained for

some parameters in the analysis of data set 1 was relatively low, we increased the length of individual chains to 1005000 samples that were collected after allowing for a burn-in period of 5000 iterations. From then, 1/100 iterations were kept.

Secondly, we analysed raw SaO phenotypes, and the (fixed) effects of hatch week (133 levels) and age of dam at laying (9 levels) were sampled together with the parameters described above in order to assess how pre-adjustment of phenotypes affected the results (we will refer to this analysis as LONG). In further analysis of dataset 2 these fixed effects were also sampled. We then repeated the analysis including in the pedigree only birds with SaO records and their ancestors (this analysis will be referred to as SHORT). Proceeding in this way speeded up the analysis considerably, but genotype probabilities could not be obtained for all birds in the original pedigree. Lastly –and using the (SHORT) pedigree described above- we carried out an analysis of Ln(100-SaO) (TRANS). This transformation reduced the skewness of the phenotypic distribution. Segregation analysis is sensitive to deviations from normality: non-genetic skewness could be the cause of spurious detection of locus with large effect. On the other hand, data transformation could remove evidence for genuine loci with large effect.

Data set 3: We carried out the analysis of data set 3 using the same pedigree as in SHORT and adding the extra generation of birds with data to the pedigree. Non-genetic fixed effects (hatch week (148 levels), age of dam at laying (21 levels) and sex (2 levels)) were sampled.

3.2.2.2 Post segregation analyses investigations

3.2.2.2.1 Sampled genotype probabilities and allele frequencies

Some exploratory analyses were carried out on genotype probabilities obtained from data sets 2 and 3 analyses. From each iteration of the Gibbs sampler, genotype configurations were obtained for all birds in the pedigree. Averaging over all iterations, probabilities of each bird being *BB*, *Bb* or *bb* could be obtained. For each bird, as many sets of genotype probabilities as chains were produced and an overall estimate of each genotype

probability was obtained by averaging the results from each chain. Genotypic frequencies at a given moment in time could be obtained by averaging the frequencies of birds in the chosen period and estimates of major allele frequencies can be obtained as $p_B = p_{BB} + 0.5\, p_{Bb}$ and $p_b = p_{bb} + 0.5\, p_{Bb}$. Since genotype probability estimates are a function of the individual phenotypic record and information from its relatives, we divided the population on the basis of the amount of information available for each individual in birds with no record, selection candidates and sires. Most of the results presented will be for the two last categories since accuracy of estimates should be highest for sires but using estimates from selection candidates as well one can attain a compromise between accuracy of estimates and sample size. Correlations amongst genotype probabilities from different analyses were obtained.

3.2.2.2.2 Estimation of putative locus effect on weight and fleshing score

In order to investigate the effect of the putative major locus on body weight and fleshing score measured at five weeks of age, the phenotypic values for these traits were regressed on functions of the genotype probabilities estimated from the segregation analysis. The model used was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Sc} + \mathbf{Rg} + \mathbf{e} \qquad [3]$$

with elements defined as in [1] and **c**, **S**, **g** and **R** are respectively the vector of random maternal environmental effects for Weight and Flesh and the design matrix relating maternal environmental effects to observations ($\mathbf{c} \sim N(0, \mathbf{I}\sigma_{em}^2)$) and the vector of random maternal genetic effects for Weight and Flesh and the design matrix relating maternal environmental effects to observations ($\mathbf{g} \sim N(0, \mathbf{A}\sigma_{gm}^2)$). **X** now includes $c_a = (p_{BB} - p_{bb})$ and $c_d = p_{Bb}$, allowing one to estimate respectively the additive and the dominance effect of the putative locus on the traits. The analysis was done within a Restricted Maximum Likelihood (REML) framework fitting a trivariate animal model using ASREML (Gilmour *et al.*, 2000).

3.3 Results

3.3.1 Description of data

A brief description of the pedigree and data structure for all data sets used is presented in Table 3.1. For all data sets, the mean number of birds with SaO record per full-sib family was less than five and the mean paternal half-sib family size was less than 30. The 70 new sire families added to data set 2 to create data set 3 had on average 51 paternal half sibs, with a range from one to 129 sibs per family, and full sib families had a mean size of eight, ranging from one to 24 offspring.

Table 3.1. Structure of oxygen saturation (SaO) data for all data sets. In some cases, information on body weight (Weight) and fleshing score (Flesh) is also presented.

	Data set 1	Data set 2	Data set 3
Birds with record for SaO (Weight and Flesh)	11919 (118782)	13450 (91761)	17066
Number of sires/paternal half-sib families with record for SaO (Weight and Flesh)	667 (755)	510 (554)	580
Mean [minimum and maximum] number of birds with record for SaO per paternal half-sib family	18 [1-77]	26 [1-106]	29 [1-129]
Number of dams with progeny with record for SaO (Weight and Flesh)	3548 (4809)	3137 (4004)	3558
Number of full-sib families with record for SaO	4130	3524	3959
Mean [minimum and maximum] number of birds with record for SaO per full-sib family	2.9 [1-23]	3.8 [1-25]	4.3 [1-25]

Table 3.2 shows descriptive statistics of the distributions of SaO and, in some cases, Weight and Flesh. Means and standard deviations were obtained with GENSTAT (GENSTAT 5 COMMITTEE, 1993). Skewness and kurtosis coefficients were obtained with MINITAB 12 (MINITAB Inc., 1998). Whilst mean body weight for data set 1 was 20% higher than mean data set 2 body weight, differences were smaller for mean SaO and Flesh (respectively, 2% lower and 2% higher for data taken at six weeks in the less recent pedigree). Coefficients of variation were similar within traits across data sets.

Table 3.2. Means and standard deviations (in brackets) for blood oxygen saturation (SaO, in % units), body weight (Weight, in decagrams (dag)) and fleshing score (Flesh, in arbitrary units, measured in a scale of 1 to 5) for unadjusted phenotypes for data sets 1 and 2, and for SaO for data set 3. Means and standard deviations of adjusted phenotypes (ADJ) are presented for SaO for data sets 1 and 2 and for Ln(100-SaO) (TRANS) for data set 2. Skewness (Sk) and kurtosis (Ku) coefficients of the distributions of raw SaO phenotypes and analysed data are also presented.

	SaO (%)	Weight (dag)	Flesh (units)	Sk	Ku
Data set 1	80.02 (9.15)	271.60 (33.66)	3.17 (0.92)	-0.78	0.46
ADJ (Data set 1)	0.64 (8.08)	-	-	-0.75	1.06
Data set 2	81.81 (7.98)	216.1 (27.98)	3.10 (0.90)	-0.85	0.88
ADJ (Data set 2)	-0.42 (7.23)	-	-	-0.74	0.93
TRANS (Data set 2)	2.80 (0.45)	-	-	-0.29	-0.17
Data set 3	81.82 (8.11)	-	-	-0.91	1.04

Untransformed (adjusted or unadjusted) SaO distributions showed skewness coefficients that were less than –0.70 for all three data sets. Adjusting raw data for fixed effects generally slightly decreased absolute skewness but increased kurtosis (making the distribution more leptokurtic), more markedly so for data set 1. Transformed data were less skewed to the left and their distribution was slightly platykurtic before adjustment for fixed effects.

Table 3.3 shows estimates of heritabilities and genetic correlations (and standard errors) for SaO, Weight and Flesh obtained from datasets 1 and 2 and heritabilities for SaO for all other data sets obtained when fitting a purely polygenic model. Except for estimates presented for data sets 1 and 2 that were obtained from trivariate analysis using full pedigrees, all estimates presented were obtained from univariate analysis.

Table 3.3. Heritabilities (on diagonal) and genetic correlations (below diagonal) and their standard errors (in brackets), obtained for body weight (Weight), fleshing score (Flesh) and blood oxygen saturation (SaO) estimated from data set 1 (traits measured at six weeks) and data set 2 (traits measured at five weeks) using full pedigrees. Only heritabilities are shown for data sets analysed using incomplete pedigrees, i.e., data set 2 (SHORT), analysis of transformed phenotypes (TRANS (Data set 2) and data set 3.

		SaO	Weight	Flesh
Data set 1	SaO	0.21 (0.02)		
	Weight	-0.02 (0.06)	0.26 (0.01)	
	Flesh	-0.10 (0.05)	0.53 (0.03)	0.22 (0.01)
Data set 2	SaO	0.15 (0.02)		
	Weight	-0.10 (0.06)	0.32 (0.01)	
	Flesh	-0.10 (0.06)	0.62 (0.02)	0.19 (0.01)
Data set 2 (SHORT)	SaO	0.15 (0.02)	-	-
TRANS (Data set 2)	Ln(100-SaO)	0.14 (0.02)	-	-
Data set 3	SaO	0.17 (0.02)	-	-

Table 3.4 shows estimates of σ_u^2, σ_e^2 and σ_p^2 obtained from the above-mentioned analyses for blood oxygen saturation for all data sets studied. Estimates from data set 1 and 2 do not refer to the same base population and records were taken at different ages. The estimated heritability for SaO from data set 1 was higher than that from data set 2. This was due to a 43% decrease in σ_u^2 accompanied by a smaller (16%) decrease in σ_e^2. Genetic correlations between SaO and Weight and Flesh were negative but not significantly different from 0 ($p>0.05$) for either data set. The heritability and variances estimated from data set 3 were similar to the estimates from data set 2, and so was the heritability of Ln(100-SaO).

Table 3.4. Estimates of genetic (σ_u^2), residual (σ_e^2) and phenotypic (σ_p^2) variances obtained for blood oxygen saturation from all data sets studied.

	σ_u^2	σ_e^2	σ_p^2
Data set 1	14.37	54.06	68.43
ADJ (Data set 2)	8.18	45.18	53.36
Data set 2 (LONG)	8.18	45.18	53.36
Data set 2 (SHORT)	8.19	45.14	53.33
TRANS (Data set 2)	2.48×10^{-2}	1.47×10^{-1}	0.17×10^{-1}
Data set 3	9.96	47.4	57.36

3.3.2 Statistical analyses

3.3.2.1 Segregation analyses

3.3.2.1.1 Data set 1

Figures 3.1 and 3.2 are a graphical summary of the properties of one of the six resulting chains, taking the samples of the major locus additive effect as an example. The trends observed for this chain and parameter were similar to the ones observed for other chains and parameters: after a 5000 iteration burn-in period the chain seemed to have converged to its equilibrium distribution and sample autocorrelation was generally low for lags over 100 for all parameters.

Figure 3.1. Samples from the additive effect of the putative major locus (*a*) plotted over time from iteration 5001 (2500 samples, taken from iteration 5000, keeping 1/100).

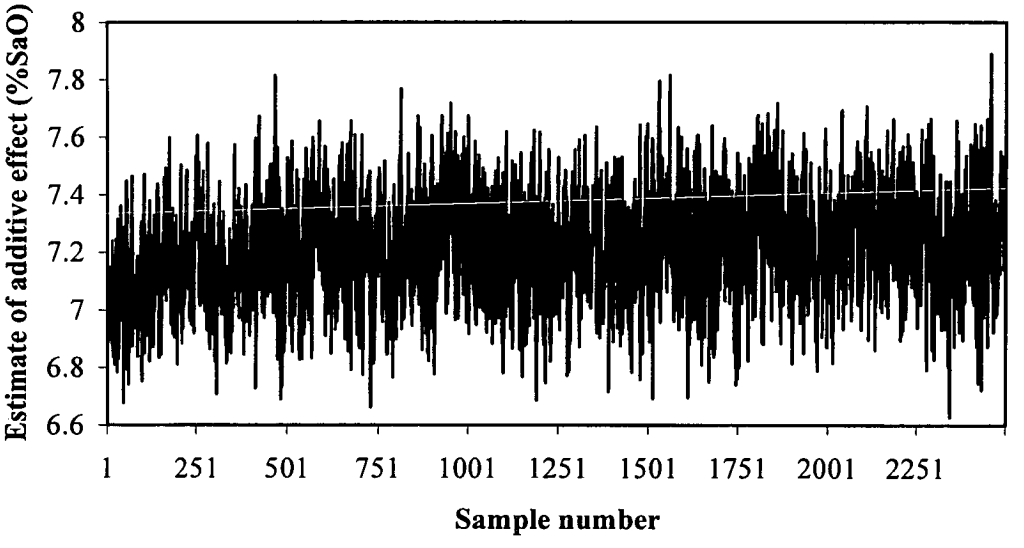
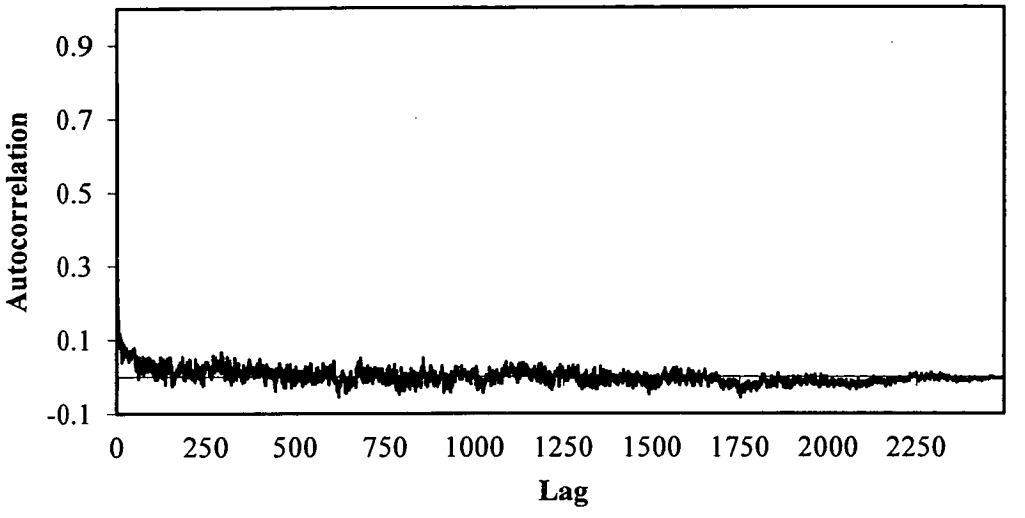


Figure 3.2. Correlogram for the 2500 samples of the additive effect of the major locus from a chain (2500 samples, taken from iteration 5000, keeping 1/100).



Marginal posterior distributions of all parameters were symmetric and approximated normal distributions. Figure 3.3 shows the marginal posterior distributions for the six chains run for the major locus additive effect.

Figure 3.3. Marginal posterior distributions for the six chains run for the major locus additive effect (*a*).

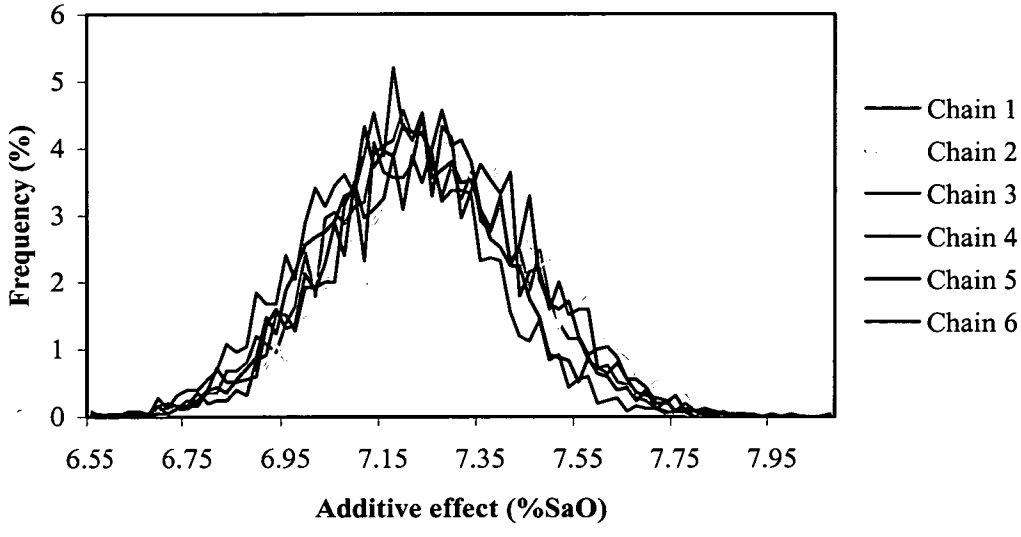


Table 3.5 shows the marginal posterior means, posterior standard deviations, Monte Carlo standard deviation of marginal posterior means and the effective number of samples per chain for all sampled parameters. The difference between marginal posterior means from the six chains was not always strictly within the Monte Carlo sampling error estimated as proposed by Geyer (1992). Nonetheless, all chains seemed to have converged to the equilibrium distribution (using different starting values) and marginal posterior means were very close. The effective number of samples was variable within chains between parameters and within parameters between chains, with values ranging from nine to more than 600 independent samples. Generally σ_u^2 and σ_e^2 showed smaller effective numbers of samples, which reflects poorer mixing for these parameters.

Table 3.5. Descriptive statistics of chains obtained from the analysis of data set 1. Marginal posterior means (MPM), posterior standard deviations (PSD), Monte Carlo standard deviations (MCSD) of MPMs and effective number of independent samples (ENS) per chain for the major locus additive (*a*) and dominance (*d*) effect, *B* allele frequency (p_B), population mean (Mean) and residual (σ_e^2) and polygenic (σ_u^2) variances. A mean ENS is also shown for each sampled parameter.

		a	*d*	p_B	Mean	σ_e^2	σ_u^2
MPM	*Chain 1*	7.236	8.089	0.528	-5.642	35.679	5.990
	Chain 2	7.201	8.049	0.524	-5.585	35.320	6.185
	Chain 3	7.241	8.034	0.524	-5.587	35.584	6.232
	Chain 4	7.158	8.113	0.526	-5.594	35.553	6.092
	Chain 5	7.265	8.011	0.527	-5.597	35.817	5.775
	Chain 6	7.214	8.025	0.525	-5.597	35.550	6.062
PSD	*Chain 1*	0.205	0.321	0.026	0.339	1.213	1.037
	Chain 2	0.192	0.315	0.026	0.338	1.029	0.939
	Chain 3	0.201	0.318	0.026	0.345	1.181	0.988
	Chain 4	0.187	0.323	0.026	0.322	1.080	0.961
	Chain 5	0.203	0.313	0.026	0.331	1.139	0.926
	Chain 6	0.194	0.321	0.026	0.327	1.142	1.040
MCSD	*Chain 1*	0.053	0.032	0.002	0.045	0.409	0.200
	Chain 2	0.011	0.037	0.001	0.038	0.110	0.141
	Chain 3	0.042	0.027	0.001	0.033	0.241	0.216
	Chain 4	0.013	0.046	0.001	0.035	0.162	0.259
	Chain 5	0.040	0.024	0.001	0.028	0.321	0.172
	Chain 6	0.016	0.023	0.001	0.026	0.213	0.172
ENS	*Chain 1*	15	102	138	56	9	27
	Chain 2	310	72	421	81	87	44
	Chain 3	23	143	410	109	24	21
	Chain 4	205	49	607	83	45	14
	Chain 5	25	174	516	137	13	29
	Chain 6	143	194	583	163	29	37
Mean ENS		120	122	446	105	34	29

Samples were pooled across chains and the means of the pooled distributions and their standard deviations were used as point estimates of the sampled parameters and their standard errors. Since the total number of independent samples for any parameter was greater than 100, the mean and the standard deviation of the pooled posterior distribution were assumed to be good estimates of the parameter and its standard error. Table 3.6 shows point estimates of the parameters sampled and those derived and the standard deviation of their pooled posterior distributions.

Table 3.6. Point estimates and standard deviations (in brackets) of the major locus additive (*a*) and dominance (*d*) effect, B allele frequency (p_B), population mean (Mean) and residual (σ_e^2) and polygenic (σ_u^2) variances obtained from the complex segregation analysis of SaO for all data analysed. Estimates are also presented for the major locus variance (σ_m^2), total phenotypic variance (σ_p^2), variance ratios (h_T, h_m, h_{am} and h) and dominance deviance (*d/a*). For TRANS (Data Set 2) standard deviations are presented for distributions including all samples or ignoring the first 267 samples of chain 5 (in grey).

	a	*d*	p_B	Mean	σ_e^2	σ_u^2	σ_m^2	σ_p^2	h_T	h_m	h_{am}	h	*d/a*
ADJ	7.22	8.05	0.53	-5.60	35.58	6.06	39.19	80.83	0.56	0.48	0.36	0.14	1.12
(Data set 1)	(0.20)	(0.32)	(0.03)	(0.33)	(1.14)	(1.00)	(3.10)	(3.07)	(0.02)	(0.02)	(0.03)	(0.02)	(0.06)
ADJ	6.46	6.60	0.65	-4.84	32.67	3.93	18.25	54.85	0.40	0.33	0.17	0.11	1.02
(Data set 2)	(0.21)	(0.32)	(0.02)	(0.26)	(0.85)	(0.62)	(1.82)	(1.84)	(0.02)	(0.02)	(0.02)	(0.02)	(0.06)
Data set 2	6.85	6.67	0.63	-4.65	32.96	3.21	21.46	57.64	0.43	0.37	0.21	0.09	0.98
(LONG)	(0.21)	(0.30)	(0.02)	(0.86)	(0.84)	(0.59)	(2.01)	(2.00)	(0.02)	(0.02)	(0.02)	(0.02)	(0.06)
Data set 2	6.84	6.67	0.63	-4.64	32.96	3.17	21.74	57.88	0.43	0.37	0.21	0.09	0.98
(SHORT)	(0.22)	(0.31)	(0.02)	(0.84)	(0.90)	(0.63)	(2.04)	(2.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.06)
TRANS	0.24	0.28	0.69	-0.23	13.28×10^{-2}	1.77×10^{-2}	2.25×10^{-2}	17.31×10^{-2}	0.23	0.13	0.05	0.12	1.17
(Data set 2)	(0.03)	(0.05)	(0.05)	(0.06)	(0.38×10^{-2})	(0.31×10^{-2})	(0.47×10^{-2})	(0.38×10^{-2})	(0.02)	(0.03)	(0.02)	(0.02)	(0.17)
	(0.02)	(0.03)	(0.04)	(0.05)	(0.38×10^{-2})	(0.31×10^{-2})	(0.46×10^{-2})	(0.38×10^{-2})	(0.02)	(0.02)	(0.02)	(0.02)	(0.17)
Data set 3	7.48	7.26	0.66	-6.49	32.93	4.20	22.64	59.77	0.45	0.38	0.20	0.11	0.97
	(0.18)	(0.25)	(0.02)	(1.12)	(0.71)	(0.61)	(2.23)	(2.20)	(0.02)	(0.02)	(0.02)	(0.02)	(0.05)

$\sigma_m^2 = 2 p_B (1 - p_B) [a + d((1 - p_B) - p_B)]^2 + [2 p_B (1 - p_B) d]^2$

$\sigma_p^2 = \sigma_m^2 + \sigma_u^2 + \sigma_e^2$

$h_T = (\sigma_m^2 + \sigma_u^2) / \sigma_p^2$

$h_m = \sigma_m^2 / \sigma_p^2$

$h_{am} = (2 p_B (1 - p_B) [a + d((1 - p_B) - p_B)]^2) / \sigma_p^2$

$h = \sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$

Figures 3.4 and 3.5 show respectively the pooled posterior distributions of σ_u^2, σ_m^2 and σ_e^2 and that of *a* and *d*. All distributions presented zero densities for parameter values equal to zero. Following Janss *et al.* (1995) we inferred that a locus with large effect on SaO was segregating in the population studied. Although the estimated *d* was slightly larger than *a*, estimated major locus effects were similar in size (the dominance deviance was indeed just different from one). We will assume in the following that *a* and *d* can be considered equal, so that the locus acts in a dominant fashion with $a = d = 7.2$ %SaO. It follows that the difference between *bb* birds and *BB* or *Bb* birds in SaO would be around 14 %. The standardised locus additive effect was 0.80 σ_p (or 1.12 $\sqrt{(\sigma_u^2 + \sigma_e^2)}$ or 1.21 σ_e). The frequency of the major locus allele that increases SaO was estimated to be $p_B = 0.53$. This locus alone would explain 48% of the total variance and 87% of the total genetic variance. The additive genetic variance accounted for by the major locus would be 79% of the total additive genetic variance.

Figure 3.4. Pooled posterior distributions of the polygenic (σ_u^2), major locus (σ_m^2) and residual (σ_e^2) variances obtained from data set 1.

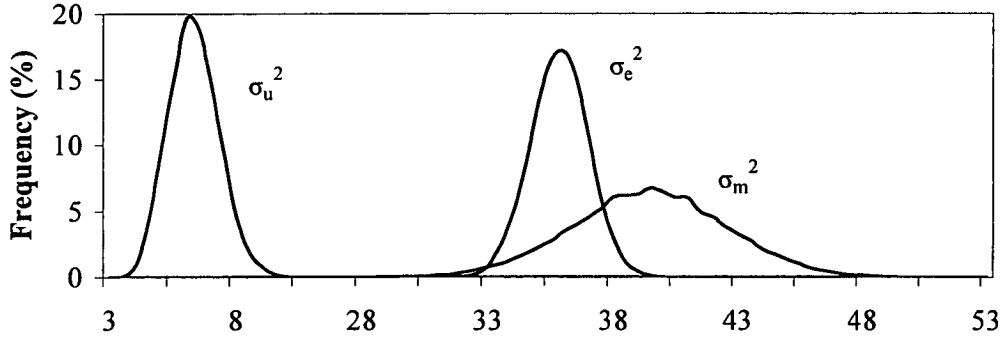
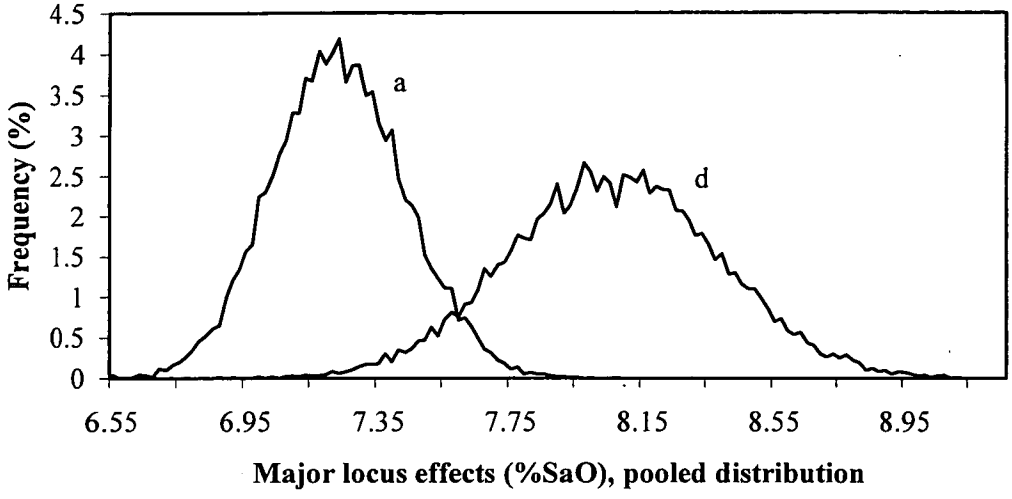


Figure 3.5. Pooled posterior distributions of the major locus additive (*a*) and dominance (d) effects obtained from data set 1.



3.3.2.1.2 Data set 2

Table 3.6 shows the means of the pooled distributions and their standard deviations for the sampled and derived parameters for all analyses.

Analysis of ADJ (data set 2): The visual inspection of the six chains did not show convergence problems. Pooled posterior distributions for all parameters presented zero densities for parameter values equal to zero. Since the density at zero for σ_m^2 was zero, presence of a locus with large effect on SaO segregating in the population studied was inferred. Figures 3.6 and 3.7 show respectively the pooled posterior distributions of *a* and *d* an of p_B for data sets 1 and 2. The estimated dominance deviance was not different from one, so the putative locus was assumed to act in a dominant fashion. The estimated locus effects were roughly the same, although slightly smaller, than the estimates obtained from the analysis of data set 1: the estimate of *a* from dataset 2 was 0.89 the estimate from data set 1 and the estimated *d* was 0.82 the estimate from data set 1. Despite this, the standardised locus additive effect increased from 0.80 to 0.87 σ_p. The estimated p_B was 0.65, that is 0.12 higher than the estimate obtained from data set 1.This major locus would explain 33% of the

total variance observed for SaO in data set 2 and 82% of the total genetic variance. The additive genetic variance accounted for by the major locus was estimated to be 70% of the total additive genetic variance.

Figure 3.6. Pooled posterior distributions of the major locus additive and dominance effects obtained from data set 1 ($a(1)$ and $d(1)$) and from data set 2 ($a(2)$ and $d(2)$).

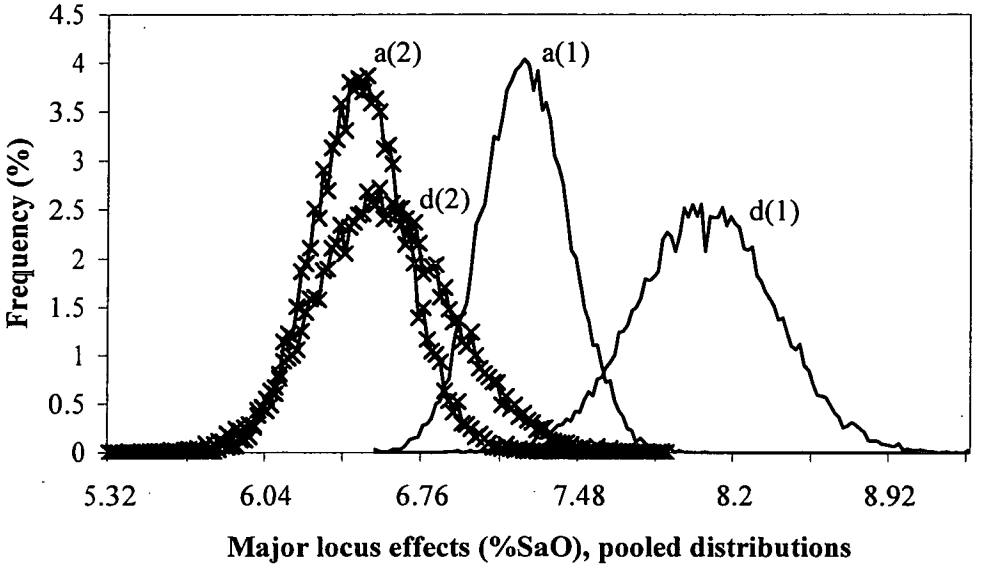
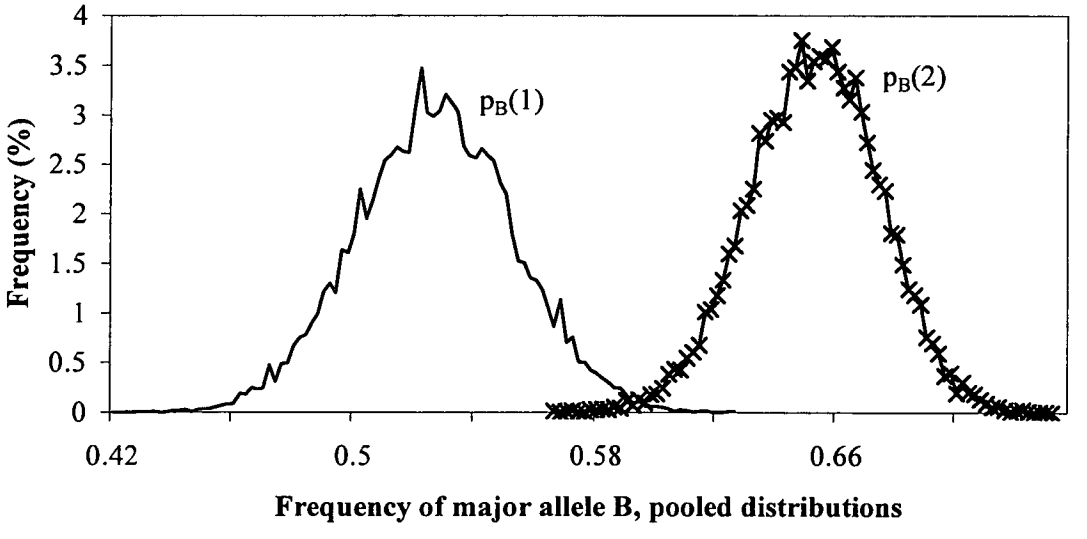


Figure 3.7. Pooled posterior distributions of the major allele B frequency obtained from data set 1 ($p_B(1)$) and from data set 2 ($p_B(2)$).

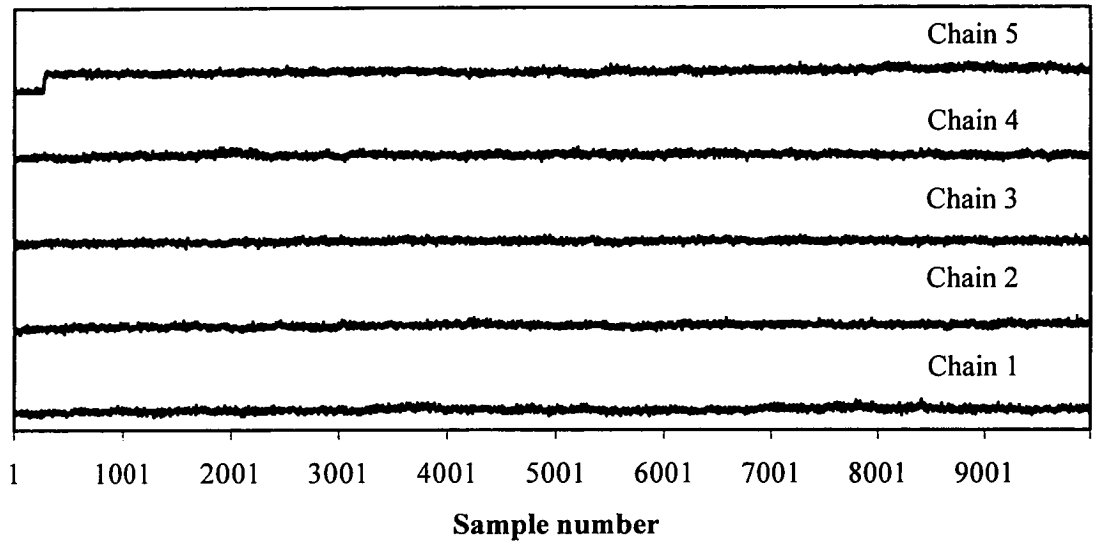


Analysis of data set 2 (LONG): The visual inspection of the five chains run did not reveal convergence problems. Table 3.6 shows that pre-adjustment of data had, for this particular data set, little effect on parameter estimates. Although slight variations in major locus effects and p_B caused a 15% difference in σ_m^2, the pooled posterior distributions of this parameter overlapped and overall, the conclusions from this analysis were not different from those drawn from ADJ (data set 2).

Analysis of data set 2 (SHORT): No convergence problems were spotted by the visual inspection of the five chains run. Parameter estimates (see Table 3.6) were generally identical to the ones obtained for data set 2 (LONG), with only small differences for, for example σ_u^2 and σ_m^2, that were in any case well within one standard deviation of the parameter's pooled posterior distribution.

Analysis of TRANS (data set 2): The visual inspection of individual chains revealed that one of the chains run visited a region of the parameter space not visited by other chains. Figure 3.8 shows samples of the additive effect of the putative major locus from the analysis of transformed phenotypes plotted against iteration number for each chain. The y-axis is in arbitrary units so the individual chains can be distinguished.

Figure 3.8. Chains obtained for the additive effect of the putative major locus (*a*) from the analysis of transformed phenotypes (Ln(100-SaO)). For each chain, the sampled *a* is plotted over time from iteration 5001 (10000 samples, taken from iteration 5000, keeping 1/100). Y-axis is in arbitrary units.



While chains 1 to 4 seem to reach the assumed equilibrium distribution from sample 1 (*i.e.*, iteration 5001), chain 5 did not converge to this distribution until sample 267 (*i.e.*, iteration 31701). Whether this was the result of a too short burn-in period or it genuinely reflects another less likely parameter configuration is not clear. Marginal posterior means and posterior standard deviations per chain for a series of parameters are presented in Table 3.7 together with the minimum (MIN) and maximum (MAX) value sampled for each parameter. All 10000 samples per chain were used to obtain the statistics shown in this table. The first 267 samples little impact on the marginal posterior means of parameters but had some effect on the dispersion of the marginal posterior distributions of the population mean, major locus effects, *B* allele frequency and σ_m^2, that was up to two-fold greater for chain 5 when including these first 267 samples.

Samples were pooled across chains and the means of the pooled distributions and their standard deviations (including chain 5 first 267 samples and excluding them) are shown in Table 3.6.

Table 3.7. Descriptive statistics of chains obtained from the analysis of transformed data. Marginal posterior means (MPM), posterior standard deviations (PSD) and minimum and maximum sampled values (MIN and MAX) for the major locus additive (*a*) and dominance (*d*) effect, *B* allele frequency (p_B), population mean (Mean), residual (σ_e^2) and polygenic (σ_u^2) variances, major locus variance (σ_m^2), total phenotypic variance (σ_p^2), variance ratios (h_T, h_m, h_{am} and h) and dominance deviance (*d*/*a*).

		a	*d*	p_B	Mean	σ_e^2	σ_u^2	σ_m^2	σ_p^2	h_T	h_m	h_{am}	h	*d*/*a*
MPM	*Chain 1*	0.242	0.287	0.697	-0.233	0.133	0.018	0.022	0.173	0.232	0.126	0.042	0.121	1.195
	Chain 2	0.242	0.266	0.670	-0.219	0.133	0.017	0.024	0.174	0.234	0.139	0.059	0.111	1.108
	Chain 3	0.234	0.275	0.673	-0.221	0.132	0.017	0.023	0.172	0.235	0.135	0.050	0.115	1.187
	Chain 4	0.248	0.291	0.709	-0.243	0.133	0.019	0.021	0.173	0.231	0.122	0.040	0.124	1.179
	Chain 5	0.241	0.267	0.687	-0.223	0.134	0.018	0.022	0.174	0.229	0.127	0.048	0.117	0.637
PSD	*Chain 1*	0.025	0.034	0.048	0.054	0.004	0.003	0.005	0.004	0.025	0.026	0.020	0.018	0.167
	Chain 2	0.023	0.030	0.041	0.054	0.004	0.003	0.004	0.004	0.024	0.023	0.020	0.018	0.158
	Chain 3	0.020	0.033	0.037	0.051	0.004	0.003	0.004	0.004	0.025	0.023	0.020	0.018	0.180
	Chain 4	0.023	0.037	0.042	0.053	0.004	0.003	0.004	0.004	0.025	0.024	0.020	0.018	0.171
	Chain 5	0.045	0.074	0.057	0.070	0.004	0.003	0.005	0.004	0.027	0.030	0.021	0.019	6.683
MIN	*Chain 1*	0.154	0.160	0.503	-0.464	0.118	0.009	0.006	0.160	0.149	0.036	0.000	0.059	0.658
	Chain 2	0.133	0.145	0.497	-0.408	0.113	0.008	0.010	0.159	0.140	0.060	0.006	0.051	0.608
	Chain 3	0.157	0.141	0.539	-0.420	0.116	0.007	0.008	0.160	0.154	0.048	0.002	0.050	0.576
	Chain 4	0.167	0.133	0.535	-0.490	0.118	0.009	0.007	0.160	0.129	0.040	0.000	0.061	0.630
	Chain 5	0.000	-0.337	0.333	-0.439	0.119	0.009	0.000	0.160	0.117	0.000	0.000	0.060	-410.842
MAX	*Chain 1*	0.359	0.439	0.868	-0.007	0.144	0.031	0.040	0.189	0.329	0.225	0.143	0.196	1.945
	Chain 2	0.348	0.371	0.804	-0.013	0.149	0.029	0.043	0.192	0.332	0.233	0.143	0.187	1.828
	Chain 3	0.305	0.399	0.816	-0.020	0.146	0.030	0.040	0.189	0.338	0.226	0.141	0.194	2.074
	Chain 4	0.346	0.445	0.869	-0.061	0.147	0.031	0.042	0.192	0.334	0.231	0.135	0.195	2.020
	Chain 5	0.344	0.408	0.835	0.210	0.152	0.032	0.042	0.191	0.322	0.225	0.138	0.190	7.960

Figures 3.9, 3.10 and 3.11 show respectively the pooled posterior distributions of σ_u^2, σ_m^2 and σ_e^2, a and d and p_B. All 50000 samples were used to plot the distributions. The figures show that the effect of including all chain 5 samples in the dispersion of major locus parameters was most noticeable for d and p_B. The distribution of σ_m^2 had a density of zero for σ_m^2 equal to zero. The smallest value of σ_m^2 was 10^{-5}. The ratio of densities between the intervals containing the most frequently sampled value of σ_m^2 ([0.02175-0.02250]) and the interval containing its smallest value ([0.00000-0.00075]) was around 550. Presence of a locus with large effect on Ln(100-SaO) was therefore inferred.

Figure 3.9. Pooled posterior distributions of the polygenic (σ_u^2), major locus (σ_m^2) and residual (σ_e^2) variances obtained from data set 1.

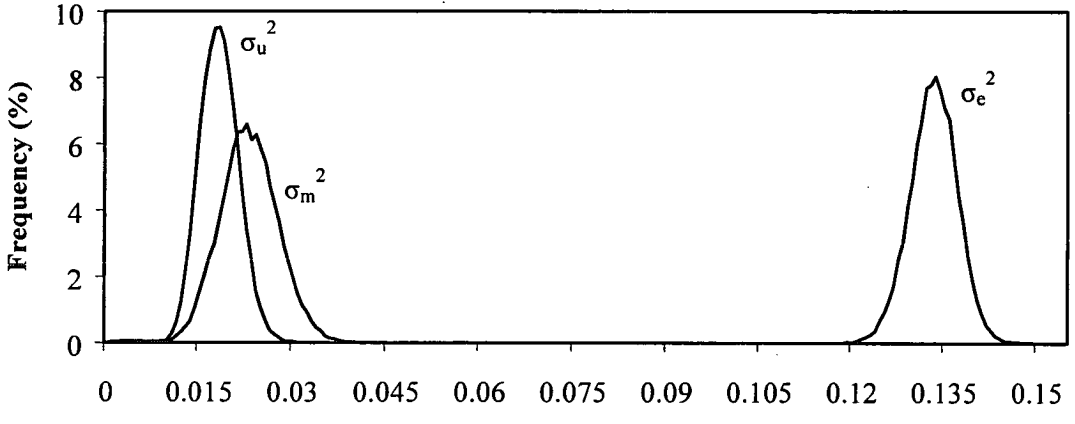


Figure 3.10. Pooled posterior distributions of the major locus additive (*a*) and dominance (*d*) effects obtained from the analysis of transformed phenotypes (Ln(100-Sa0)).

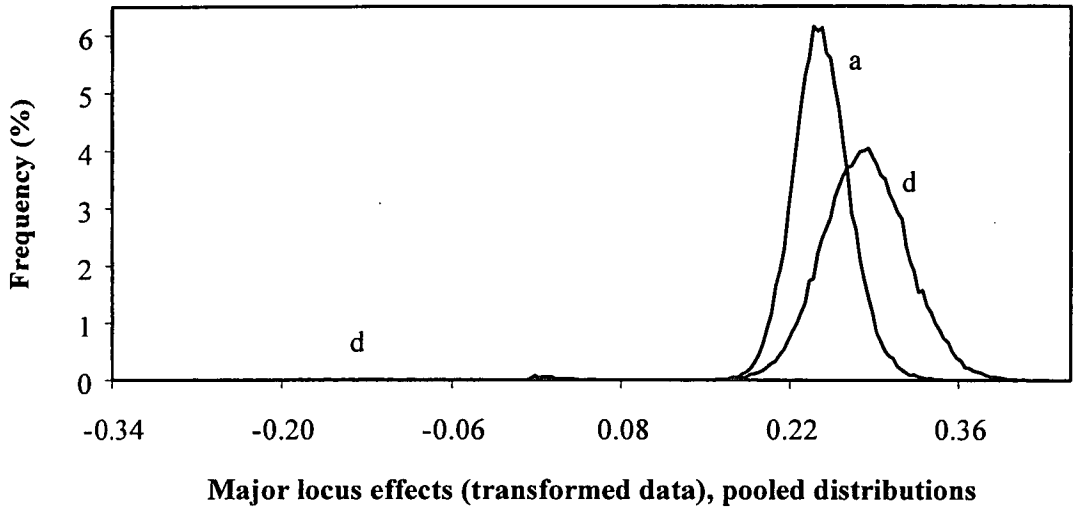
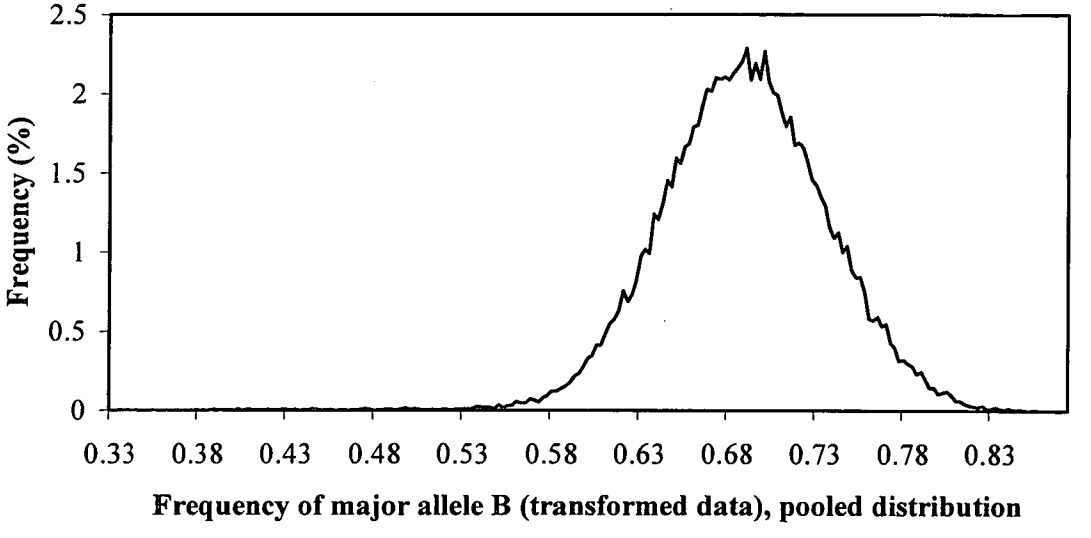


Figure 3.11. Pooled posterior distribution of the major allele B frequency obtained from the analysis of transformed phenotypes (Ln(100-Sa0)).



Similarly to the untransformed SaO phenotypes analyses results, the dominance deviance was not different from one for Ln(100-SaO). The estimated locus effects were roughly $a = d = 0.24$ Ln(100–SaO), which translates to 0.58 σ_p, that is around 36% smaller

than for SaO. The frequency of the major locus allele that increases Ln(100–SaO) was estimated to be $p_B = 0.69$. This locus alone would explain 13% of the total variance and 55% of the total genetic variance. The additive variance accounted for by the major locus would be 31% of the total estimated additive variance that was 15% of the total variance.

3.3.2.1.3 Data set 3

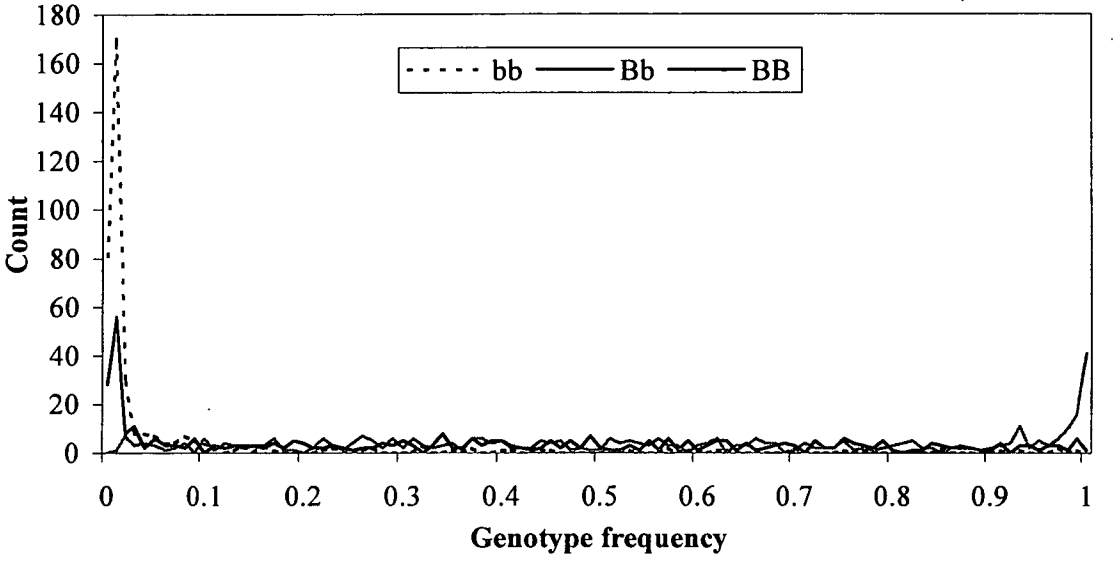
No convergence problems were spotted by the visual inspection of the five chains run. Parameter estimates (see Table 3.6) were similar to the ones obtained for untransformed data set 2 analyses, with slightly a higher standardised additive effect (0.97 σ_p) and $p_B = 0.66$. The total additive heritability was 0.27 and the major locus contributed 74% of the total additive variance and 84% of the genetic variance.

3.3.2.2 Post segregation analyses investigations

3.3.2.2.1 Sampled genotype probabilities and allele frequencies

The correlation of estimated probabilities for each genotype between chains was higher than 0.96 for all analyses of untransformed data and higher than 0.84 for the analysis of transformed data, and the mean standard error (averaging over all birds with data and ancestors) of each genotype probability was smaller than 0.01. Figure 3.12 shows the distribution of genotype probabilities for all the sires obtained for ADJ (data set 2).

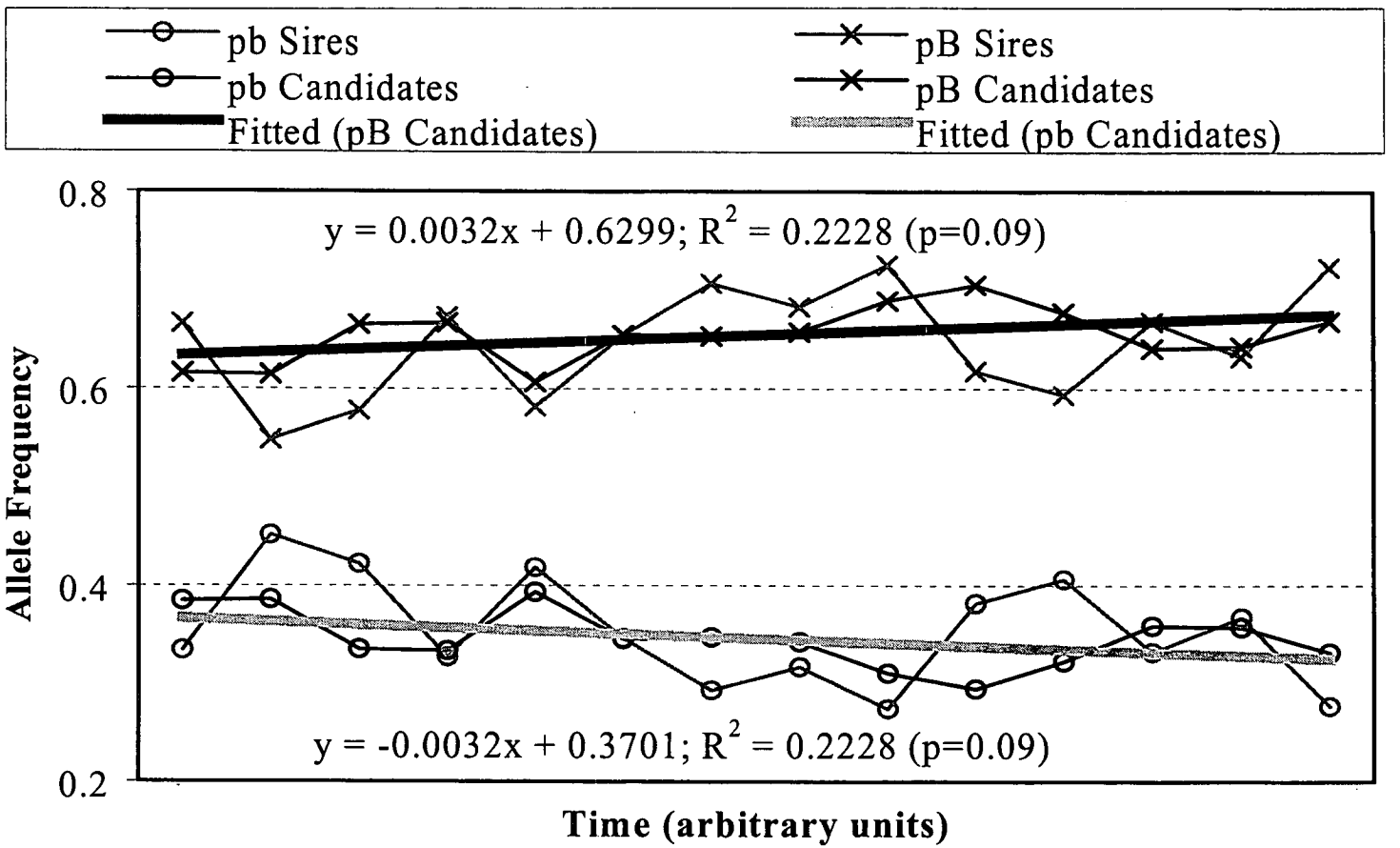
Figure 3.12. Distribution of genotype probabilities for all the sires obtained for ADJ (data set 2).



Sire genotype probabilities obtained from ADJ (data set 2) show that approximately 1.8% of the sires were assigned a *bb* genotype, 32.9% a *Bb* genotype and 11.1% a *BB* genotype with a probability higher than 0.8. If the inferred mode of action of the putative gene is dominant, information from relatives is necessary to discriminate heterozygotes from the dominant homozygotes. In our case, more than 40% of sires were identified as being either *BB* or *Bb* with high probability (>0.8) but around a further 15% of sires had similar (and close to 0.5) probabilities of being *BB* or *Bb*, although they had very low probabilities of being *bb*.

Figure 3.13 shows how major allele frequencies vary over time. Estimates of allele frequencies were obtained from sires alone and from all birds with records. For sires, each point in the graph was obtained from 29 birds whereas in the case of selection candidates each point was obtained from 1000 birds. For selection candidates, regression of allele frequencies over time has a negative slope for p_b and a positive one for p_B, showing how within the period corresponding to data set 2 the frequency of the major allele that increases SaO increases over time.

Figure 3.13. Major allele frequencies plotted over time. Estimates of allele frequencies were obtained from sires alone and from all birds with records.



Correlations between genotype probabilities obtained from ADJ (data set 2), Data set 2 (LONG) and Data set 2 (SHORT) for sires and selection candidates were of the order of 0.90.

Data added to data set 2 to create data set 3 (*i.e.*, large progeny groups) did not have a great impact on the genotype probability estimates of birds that had progeny on both data sets. The correlations of estimated sire probabilities, obtained from both data sets, of being either *bb*, *Bb* or *BB* were respectively 0.94, 0.95 and 0.96. On the other hand, having information on progeny had a visible impact on the genotype probabilities of birds that did not have offspring in data set 2. Adding this information allowed discrimination between *BB* and *Bb* birds. The correlations of estimated sire probabilities, obtained from both data sets, of being either *bb*, *Bb* or *BB* were respectively 0.88, 0.36 and 0.26. The estimated *pB* and *pb* from these birds were 0.64 and 0.36 respectively and the mean genotype probabilities were similar to the ones obtained from data set 2. None of these sires were assigned a *bb* genotype with a probability higher than 0.8, but 42.7% were identified as *Bb* and 4.5% as *BB* with a probability higher than 0.8.

The correlation between the probability of being heterozygote estimated from transformed and untransformed data was –0.17 ($p < 0.001$) for birds with SaO data and –0.11 ($p = 0.03$) for sires with SaO data.

3.3.2.2.2 Estimation of putative locus effect on weight and fleshing score

Table 3.8 shows the estimates of the putative locus additive (*a*) and dominance (*d*) effect on SaO, Weight and Flesh obtained from the regression of trait values on genotype probabilities at the major locus. The estimated additive effect was not different from zero for Weight and Flesh, but the estimated dominance effect was large for both. This suggests that birds heterozygous at this putative locus would have substantially higher body weight and fleshing score at 5 weeks than either of the homozygotes. The estimated effects for SaO were significantly larger than the estimates obtained from the segregation analysis.

Table 3.8. Estimates and standard errors (in brackets) of the putative locus additive (*a*) and dominance (*d*) effect for SaO, Weight and Flesh obtained from the regression of trait values on genotype probabilities at the major locus.

	a	*d*
SaO	11.96 (0.14)**	16.61 (0.27)**
Weight	-0.71 (0.55)	5.84 (0.88)**
Flesh	-0.03 (0.02)	0.24 (0.04)*

* $p<0.05$
** $p<0.01$

3.4 Discussion

Overall, the segregation analyses of line 3 SaO data seem to indicate that a locus with large effect is involved in the genetic control of SaO. Results obtained from the three sets of untransformed data (data sets 1, 2 and 3) provide a fairly consistent picture: a dominant major locus with an additive effect of around 0.90 σ_p that is responsible for a predicted difference in SaO amongst homozygous of more than 10% is segregating in line 3, and the frequency of the allele that increases SaO increases with selection on SaO from p_B = 0.53 to around 0.65. It is difficult to assess if the observed difference in estimated p_B from data sets 1 and 2 really reflects a change in allele frequencies over time or is a consequence of phenotypes recorded at different ages (six and five weeks respectively). Nonetheless, Figure 3.13 shows that, within a time period (data set 2), the estimated frequency of the allele that increases SaO increases slightly over time, which is consistent with the between data set trend. This within time period trend becomes more obvious if the last period of time is ignored, which could be due to the fact that estimates of genotype frequencies in latter periods are only based on phenotypes and information from ancestors, but not from progeny. However, it is still possible that an increase in incidence or severity of disorder with age could cause changes in the phenotypic distribution and, for instance, increase the size of the estimated gene effect and/or influence the estimated frequency of the favourable allele between data sets.

For data set 1, the predicted proportion of heterozygous individuals would be around 50%. Because the gene is dominant, around 25% of the population would show low SaO values. Despite this, this locus alone would explain 48% of the total variance and around 87% of the genetic variance in the "base population". For data set 2, the predicted proportion of heterozygous individuals would be around 45% and only around 12% of the population would show low SaO values. The proportion of the total variance observed in data set 2 "base population" explained by this putative locus has decreased to 33% and that of the genetic variance to 80%.

From data set 1 to data set 2, the estimate of the major locus variance has more than halved. Changes in the major locus allele frequency alone account for 71% of this difference compared to 46% accounted for by changes in estimated effects alone. The polygenic variance estimated from data set 2 was roughly two thirds of the estimate obtained from data set 1. This, together with an increase in the frequency of the allele that increases SaO, is consistent with selection being carried out to increase SaO in the population studied. As a result of decreases in major gene and polygenic variances, the total and the major locus heritabilities have decreased, since the residual variance has remained approximately constant.

When a major locus is segregating, the population distribution of phenotypes can be skewed. MacLean *et al.* (1975) showed that skewness of the phenotypic distributions, when not caused by segregation of loci with large effect, could lead to detection of spurious major locus. The mean skewness coefficient of our three untransformed data sets (after adjustment for fixed effects) was around –0.77. In an outbred situation, where one expects only a proportion of families to be segregating at a putative major locus, the trait distribution within full and/or half sib families would depend on the sire and dam major genotypes, and families that do not segregate at the major locus would only show this background skewness. Figure 3.14 shows the distribution of adjusted SaO phenotypes within two sire families with over 100 offspring each. It can be seen that dispersion within sire family 1 (569) is larger than

dispersion within sire family 2 (613). Sire 1 was assigned a *Bb* genotype with a probability greater than 0.99 whereas sire 2 was assigned a *BB* genotype with a probability greater than 0.98 and a *bb* genotype with a probability smaller than 0.02.

Figure 3.14. Distribution of adjusted SaO phenotypes within two sire families with over 100 offspring each

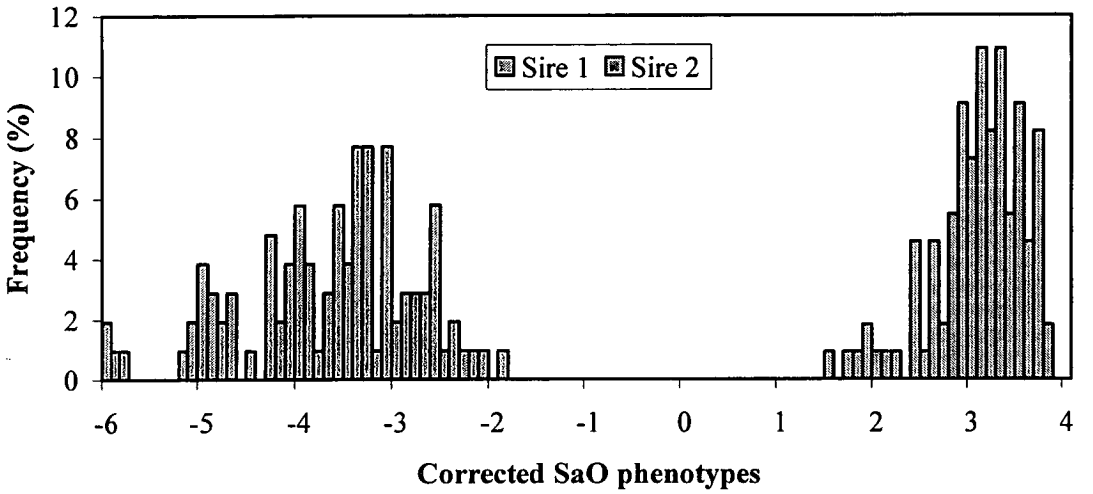
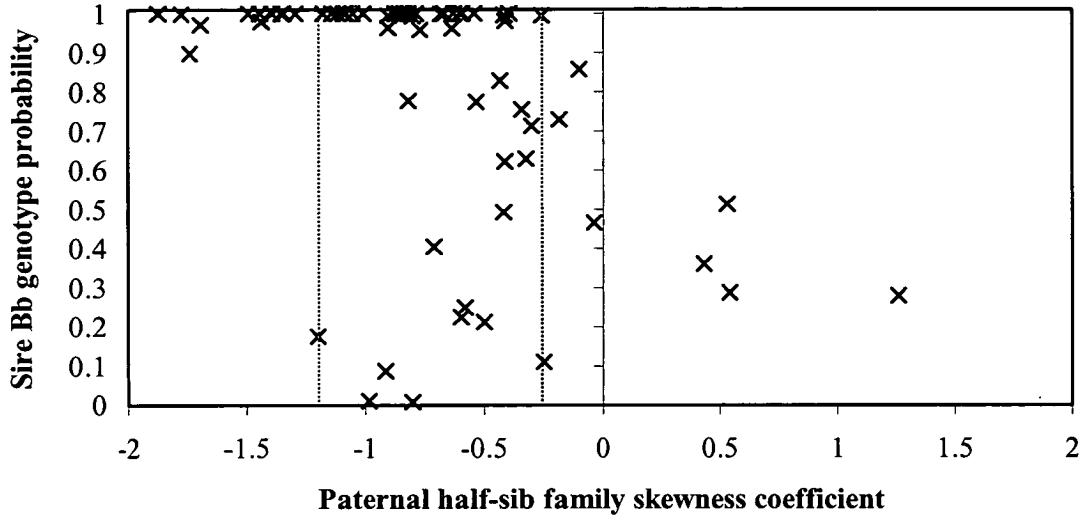


Figure 3.15 shows that all sires with progeny trait distribution with a skewness coefficient more negative than –1.2 were assigned a *Bb* genotype with a probability greater than 0.99 but for family skewness coefficients greater than –0.26, the probability of the sire being *Bb* was always less than 0.99.

Figure 3.15. Sire *Bb* genotype probability plotted against paternal half-sib family skewness coefficient.



MacLean *et al.* (1976) suggested applying a transformation to the phenotypic data to remove skewness prior to analysis, but showed that this could considerably reduce the power to detect major genes when present, as well as posing problems for the interpretation of the results (Demenais *et al.*, 1986). Although the outcome of the analysis of transformed data was that a locus with large effect on Ln(100-SaO) was segregating in line 3, neither the frequency nor the mode of action of this putative locus were in agreement with the results obtained from untransformed data. Indeed, an allele that increases Ln(100-SaO) would decrease SaO, so the estimated p_B = 0.69 from this analysis needs to be compared to (1 – 0.65) = 0.35. In the same way, if the locus that increases Ln(100-SaO) were dominant, the proportion of birds showing low SaO values would be around 81% compared with the predicted 12% from the untransformed data analysis. The fact that the estimates of p_{Bb} are not similar from transformed and untransformed data suggests that these analyses are describing different phenomena. Selection experiments carried out in other broiler populations to study ascites susceptibility tend to suggest that this trait is influenced by a single biallelic major locus that would act in a recessive fashion (see for example, Druyan *et*

al. (2001), Druyan *et al.* (2002), Wideman and French (1999) and Wideman and French (2000)). This would support the mode of action suggested for SaO by the analysis of untransformed data.

Lalouel *et al.* (1983) suggested the estimation of transmission probabilities at the major locus, jointly with all the parameters already described, to circumvent the problem of false detection due to skewness. In our analysis, the major locus was assumed to have Mendelian transmission probabilities. We conjecture that deep pedigrees available from commercial animal populations together with family structures with more family links and a larger number of sibs per family than human populations make it easier to discriminate between non-genetic and genetic skewness, and within the latter, between skewness of the polygenic distribution or due to segregation of a major locus. Szydlowski and Szwaczkowski (1998) used a multigenerational real Leghorn pedigree and simulated 4000 female phenotypes for polygenic and mixed-inheritance traits with different degrees of polygenic and non-genetic skewness. They concluded that discrimination between genetic and non-genetic skewness was possible, but that polygenic skewness greater or equal to 0.2 could lead to spurious detection of major genes. However, they inferred segregation of a locus with large effect when the ratio of the density of σ_m^2 at the mode of its posterior distribution and at zero was greater than one, which is far less stringent than the criterion we used. They also showed that non-genetic skewness led to overestimation of σ_e^2 in their simulated data sets.

Knott *et al.* (1992a) showed by simulation that genes with an effect of one within major genotype standard deviation, and explaining at least 24% of the total variance, could be detected by segregation analysis with reasonable power. In their analysis they only used half-sib data structure and they suggest that improvements in power could be achieved when using more complex pedigrees. Knott *et al.* (1992a) also showed that dominant genes or genes with one rare allele where more easily detected as they caused the phenotypic distribution to be skewed. Miyake *et al.* (1999b) simulated two-generation pedigrees of 50 sires mated to ten dams that produced one offspring each. They simulated a major locus with

effects ranging from zero to 4.5 σ_u and concluded that effects greater than 1 σ_u were detectable. They suggest that except for loci with the largest effect they simulated, the accuracy of the estimates of the major locus effects was greater when the locus was dominant. Knott *et al.* (1992b) showed that in addition estimates of polygenic values were also more accurate. Using their criterion to infer segregation of major locus, Szydlowski and Szwaczkowski (1998) were able to detect simulated major loci responsible for a third of the genetic variance and a tenth of the phenotypic variance but none of these loci would have been detected using a threshold of 20 for the σ_m^2 density ratio. The size of additive effects of major loci reported in a sample of studies carried out in livestock populations using the same methodology as in the current study (*e.g.* Janss *et al.* (1997a), Janss *et al.* (1997b), Szydlowski and Szwaczkowski (2001), Miyake *et al.* (1999a), Walling *et al.* (2002)) ranges from around 0.1 to around 1.5 σ_p (meat pH and % cooking loss in Meishan x commercial Dutch pig cross) and the proportion of phenotypic variance explained by these major loci ranges from 6% (body weight at sexual maturity in laying chickens) to 64% (% drip loss in Meishan x commercial Dutch pig cross). Among these studies, Janss *et al.* (1997a), Janss *et al.* (1997b) and Szydlowski and Szwaczkowski (2001) used the same threshold as in the current study to infer presence of a major locus. The effect of the putative locus identified in our study is well within the range of effect sizes reported, for transformed or untransformed data analyses.

Data analysed by Janss *et al.* (1997a) and Janss *et al.* (1997b) were revisited by De Koning (2001) who had also access genotypes on 127 marker loci for this population. De Koning (2001) performed a series of linkage analyses and identified various QTL for several of the traits analysed. He latter repeated the segregation analysis for fatness traits including identified QTL as covariates, in order to see if the joint effect of these QTL could explain the major loci identified by Janss *et al.* (1997b). After fitting the QTL in the model, there was still evidence for segregation of major loci, although some reduction in σ_m^2 and σ_e^2 estimates was observed. De Koning (2001) suggests that failure to detect QTL that could explain

identified QTL could be due to interactions between QTL or differences in assumptions and "tools" of segregation analysis and mapping methods. Another reason could be that, despite the experiment being carefully designed, some source of variance has not been accounted for that mimics to a certain extent segregation of a major gene. Moreover, since the pedigree used in the study of Janss and collaborators (1997a, 1997b), although relatively large, was shallow, genetic and environmental effects would have been difficult to disentangle in the same way as polygenic and major locus effects. Miyake *et al.* (1999c) used simulated data of two-generation pedigrees to show how, depending on the initial values used for major locus parameters, population mean and polygenic and residual variances, the estimates of these parameters varied, but took values such that the phenotypic distribution of a population re-created with theses estimates closely matched the phenotypic distribution of the simulated population.

We observed that, consistently for all the analyses carried out, the total additive variance estimated using a mixed inheritance model was greater than the additive variance estimated using a polygenic model. In order to assess whether this would be expected in the presence of a segregating major gene in a population under selection, a simulation study was performed. 125 five-generation pedigrees (base population and four generations of random or phenotypic selection) were simulated with a structure chosen to resemble the (real) pedigree analysed. In each generation, 40 males were mated to eight (different) females that produced three male and three female offspring each (*i.e.*, population size was maintained constant). In the case of phenotypic selection, the 40 males and 320 females with highest phenotypes were selected to produce the next generation. The simulated pedigrees consisted in 9600 individuals. Phenotypes were assumed to be under the genetic control of a gene with large effect and a large number of polygenes with small additive effect, as well as partially determined by the environment. The parameters used for the simulation were the ones presented in Table 3.6 for ADJ (data set 2). Data were analysed assuming an infinitesimal model using data from all animals or censoring phenotypes from females (that were not

available in data set 2). In a second stage, the pedigrees were analysed assuming a mixed inheritance model (for materials and methods see sections in previous chapter and this chapter). Table 3.9 shows the means and standard deviations of the parameters estimated. In brief, the simulation study showed that, under phenotypic selection, the estimate of the total additive variance is severely biased downward if a purely polygenic model is assumed. Estimates of additive variances obtained by simulation are in close agreement with results obtained when analysing real data. Assuming a change in major allele frequencies like the one shown in Figure 3.13 for selection candidates, we estimated that total additive variance would be underestimated by around 22% when assuming a purely polygenic model. Results from simulation show that this underestimation is around 31%. This, together with the fact that total additive variance seems to be well estimated (only slightly overestimated) under random selection, both assuming purely polygenic or mixed inheritance, would support the hypothesis that changes in allele frequencies caused by selection are the source of the observed discrepancies.

The results presented in chapter two showed that, for line 3, the inclusion of environmental maternal effects for SaO did not significantly ($p>0.05$) improve the fit of the model for SaO and explained only around 2 % of the total variance for data set 1 analyses. Similar results were obtained for data set 2. We nonetheless investigated the effect of including environmental maternal effects in a model including a locus with large effect, and used data set 2 (SHORT) to this aim. Given that these analyses were computer intensive, only one chain of 705000 iterations was run. This analyses showed that estimates of parameters presented in Table 3.6 for data set 2 (SHORT) were unaffected by the inclusion of environmental maternal effects, and that these explained less than 0.1 % of the total variance when a major locus was included in the model.

Table 3.9. Simulated parameters (SIM, in bold) and estimated values assuming either a purely polygenic model (POL) or a mixed inheritance model (MIX). Estimates of parameters were obtained by averaging estimates from 125 replicated populations. Standard deviations are presented in brackets. *a* is the major locus additive effect, *d* the dominance effect and , p_B the B allele frequency. Population mean (Mean) and residual (σ_e^2) and polygenic (σ_u^2) variances are also shown, together with major locus additive, dominance and total variances (σ_{am}^2, σ_{dm}^2 and σ_m^2), total phenotypic variance (σ_p^2), total additive variance (σ_a^2) and variance ratios (h_T, h and h_a). POLF shows estimates of parameters obtained from polygenic analyses before censoring female phenotypes. Results are presented for populations undergoing phenotypic (PHE) or random (RAN) selection.

		a	*d*	p_B	Mean	σ_e^2	σ_u^2	σ_{am}^2	σ_{dm}^2	σ_m^2	σ_p^2	σ_a^2	h_T	h	h_a
	SIM	**6.46**	**6.60**	**0.65**	NA	**32.67**	**3.93**	9.13	9.02	18.15	54.75	13.06	0.40	0.11	0.24
PHE	**POLF**	NA	NA	NA	NA	39.31 (1.18)	6.68 (0.48)	NA	NA	NA	45.99 (1.18)	6.68 (0.48)	NA	0.17 (0.01)	NA
	POL	NA	NA	NA	NA	37.38 (1.42)	8.94 (0.79)	NA	NA	NA	46.32 (1.36)	8.94 (0.79)	NA	0.24 (0.03)	NA
	MIX	6.77 (0.49)	5.99 (0.58)	0.66 (0.03)	-7.55 (0.57)	32.02 (1.14)	5.01 (0.68)	10.32 (2.24)	7.11 (1.20)	17.43 (2.20)	54.46 (2.16)	15.33 (1.97)	0.41 (0.03)	0.14 (0.02)	0.28 (0.03)
RAN	**POLF**	NA	NA	NA	NA	39.78 (1.41)	15.17 (2.25)	NA	NA	NA	54.95 (2.79)	15.17 (2.25)	NA	0.38 (0.06)	NA
	POL	NA	NA	NA	NA	39.63 (2.02)	15.24 (3.04)	NA	NA	NA	54.87 (3.01)	15.24 (3.04)	NA	0.39 (0.09)	NA
	MIX	6.49 (0.35)	6.42 (1.27)	0.65 (0.03)	-4.96 (0.45)	32.73 (1.48)	3.76 (1.40)	9.53 (3.35)	8.77 (1.67)	18.30 (2.82)	54.79 (3.08)	13.29 (3.45)	0.40 (0.04)	0.10 (0.04)	0.24 (0.04)

$\sigma_{am}^2 = 2\,p_B\,(1-p_B)\,[a + d\,((1-p_B) - p_B)]^2$; $\sigma_{dm}^2 = [2\,p_B\,(1-p_B)\,d]^2$; $\sigma_m^2 = \sigma_{am}^2 + \sigma_{am}^2$

$\sigma_p^2 = \sigma_m^2 + \sigma_u^2 + \sigma_e^2$; $\sigma_a^2 = \sigma_{am}^2 + \sigma_u^2$

$h_T = (\sigma_m^2 + \sigma_u^2)/\,\sigma_p^2$; $h = \sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$; $h_a = (\sigma_{am}^2 + \sigma_u^2)/\,\sigma_p^2$

NA : not available

In chapter two, we also showed that, when dividing the population into subsets of "ascitic" and "non-ascitic" birds (based on SaO readings), the within-subset estimated heritability was only 0.03 for the subset of birds with readings smaller than 75 % SaO (subset 1) and 0.04 for birds with readings greater than 80 % SaO (subset 2). Assuming a mixed inheritance model, the estimated proportion of *bb* birds for data set 1 (that is the one analysed in chapter two) was around 25 %, which is approximately the proportion of birds in subset 1. Heritabilities estimated from subsets 1 and 2 could be taken as the within major genotype (*i.e.*, polygenic) heritabilities, the heritability from subset 1 being the within *bb* genotype heritability and the one from subset 2 the within *B-* genotype heritability. The polygenic heritability defined both as a σ_u^2 proportion of $\sigma_u^2 + \sigma_e^2$ and σ_p^2 estimated from the segregation analysis of data set 1 was respectively 0.14 and 0.07, which are slightly higher than the heritabilities estimated from subsets 1 and 2 using a polygenic model. The simulation study performed in the current chapter showed, nonetheless, that both simulated $\sigma_u^2 / (\sigma_u^2 + \sigma_e^2)$ and simulated σ_u^2 / σ_p^2 are slightly overestimated when analysing the data using a mixed inheritance model. This slight overestimation was also observed for σ_u^2, and that could explain, at least partly, the difference between the estimated σ_u^2 from subsets 1 and 2 (that was around one) and the estimated σ_u^2 from the segregation analysis of data set 1 (that was around 6). These results further support the existence of major locus and polygenic variation for SaO.

Typically, when there is not enough information in the data, estimates of p_B close to 0.5 are expected - since genotypes are initialised as *Bb* -, together with large standard errors for each one of the bird's genotype probabilities. When non-genetic skewness is the cause of spurious major gene detection, one could expect that for most sires the probability of being heterozygous (p_{Bb}) should be close to 1, especially when progeny groups are large (if progeny groups are small, by chance all progeny could fall either in the high or low tail of the trait distribution). In our case some sires were assigned genotypes other than *Bb* with

high probability and standard errors of genotype probabilities estimated for each bird were generally small, since correlations of estimates obtained from different chains were high.

In our population, no dams have phenotypic information, and their genotypes are therefore inferred from pedigree information alone. It would be interesting to see what the impact of adding phenotypic information of dams is on the estimated genotype probabilities of their mates.

By regressing Weight and Flesh phenotypes on functions of major locus genotype probabilities we obtained estimates of the effects of the putative locus on these traits. The locus that has an effect on SaO seems to act in an overdominant fashion for weight and fleshing score. This would be consistent with the estimate of zero for the genetic correlation between these traits and oxygen saturation from the analysis done assuming a purely polygenic model and would also explain the intermediate frequency of the high SaO allele estimated from data set 1, which increases after selection to increase oxygen saturation. In an experiment involving the hypobaric exposure of birds, Pavlidis *et al.* (2003) observed significant heterosis for body weight at 14, 18 and 42 days in the reciprocal crosses of an ascites-resistant and an ascites-susceptible line, but observed no differences in body weight between the ascites-resistant and ascites-susceptible lines. This observations fit well with our results. Estimates of the putative gene effects for SaO obtained from this analysis were approximately two-fold the ones obtained from the segregation analysis. Genotype probabilities were estimated from segregation analysis and only birds with phenotypes from the tails of the SaO trait distribution (and/or strong family information) are likely to have extreme (*i.e.*, close to 0 or 1) estimates of genotype probabilities. This would cause these individuals to have a high influence in the regression and hence could lead to an overestimation of the locus effect for SaO. The extent to which this would affect estimates of effects for Weight and Flesh would be a function of the true genetic correlation amongst these traits and SaO.

3.5 Conclusions and further research

Our study indicates that a QTL or gene with large effect on SaO is segregating in the population studied. It must be borne in mind that, although segregation analysis is the most powerful marker-free method for major gene/QTL detection, it is sensitive to deviations from normality, and the distribution of the data analysed was skewed. Nevertheless, the majority of the different analyses performed here are consistent with the presence of a major gene. Only the different result from the analysis of transformed data provides a cautionary note, but previous studies suggest this may be expected even in the presence of a genuine major gene. Accepting the presence of a major gene, the mode of action of the putative locus on SaO and on weight and fleshing score, the fixation of the favourable allele (*i.e.*, the one that increases SaO) by means of traditional selection would be a difficult task. This is because the combined effects of selection to increase both SaO and weight and fleshing score will result in the heterozygote being the favoured genotype. Nonetheless, elimination of carriers of the allele that decreases SaO is of interest since it would lead to greatly improved broiler health and would reduce the broiler industry ascites-related economic losses. A QTL mapping study is a necessary further step that would confirm or refute our findings. In the case that our findings were confirmed, it could provide a tool to manage the putative locus allele frequencies in the population.

CHAPTER FOUR

4 A LINKAGE STUDY FOR BLOOD OXYGEN SATURATION AROUND CANDIDATE GENES

4.1 Background and introduction

A segregation analysis was carried out on blood oxygen saturation (SaO) data for a line of meat-type chickens and the results obtained suggested that a major gene with two alleles at intermediate frequencies was involved in the genetic control of SaO. In brief, we estimated that the putative major gene would account for a difference of around 13 % SaO between the homozygotes and that the decreasing major allele was recessive. These results are consistent with other data from selection experiments carried out in other broiler populations to study ascites susceptibility that suggest that this trait is influenced by a single biallelic major locus, acting in a recessive fashion (see for example, Druyan *et al.* (2001), Druyan *et al.* (2002), Wideman and French (1999) and Wideman and French (2000)). The mode of action of the putative locus on SaO together with the fact that we estimated that it had an overdominant effect on weight at five weeks and fleshing score (see chapter three) hinder allele frequency manipulation at the major locus without the use of molecular markers. In recent years the development of molecular techniques has allowed the construction of detailed linkage maps for a wide variety of species, including the chicken (Groenen *et al.*, 2000; Schmid *et al.*, 2000). Several studies have used these maps to identify marker-trait associations, for characters of economic importance, mainly growth-related (Van Kaam *et al.*, 1998; Van Kaam *et al.*, 1999; Sewalem *et al.*, 2002; Ikeobi *et al.*, 2002; De Koning *et al.*, 2003) but also health-related (Yonash *et al.*, 1999), in different mapping populations. These associations could be exploited in breeding programmes to achieve more rapid improvement, by either marker-assisted selection (MAS) or marker assisted introgression (MAI). Given the effect of ascites on welfare and production, a QTL mapping experiment was designed to identify genomic regions with an effect on SaO in the line studied. A mapping population was designed to maximise our chances to map the putative

major locus identified by the segregation analysis of SaO data. We used a candidate gene approach and chose ryanodine receptors 1, 2 and 3 (RYR1, RYR2 and RYR3) as candidate loci for our study based on prior information that suggested the involvement of RYR2 in right ventricular hypertrophy as a response to hypoxia-induced pulmonary hypertension in rats (Zhao *et al.*, 2001) and the evidence of its involvement in several cardiopathies in humans (see for example, Laitinen *et al.* (2001), Tiso *et al.* (2002)).

4.2 Materials and methods

4.2.1 Experimental design: Power study, mapping population and traits

The mapping study was to be carried out using the most recent generations of birds from the same commercial breeding line for which segregation analyses had been performed. The mapping population was designed to take advantage of the large paternal half-sib families that can be obtained in chicken populations. In the population studied, within the breeding program management conditions, the mean paternal half-sib family size achieved was around 50, with maximum values around 130. Using the terminology of Weller *et al.* (1990), a daughter-type design (*i.e.*, a standard half-sib design) was preferred to a granddaughter-type design since the former was better suited for management reasons and allowed the mapping population to be produced and phenotyped within a shorter time-span. Population size was determined following a power study (see below). The main focus of the study was on SaO, but body weight at seven days of age (7dwt) and production traits measured at five weeks of age (body weight (Weight), fleshing score (Flesh) and selection weight (Selwt, estimated as suggested by Hill (1984)) will also be studied, together with weight gain between seven days and five weeks (Gain). We also analysed a transformation of SaO (Ln(100-SaO)) that had a distribution closer to a Normal distribution than untransformed data. Results from previous chapters suggest that additive genetic (due to polygenes and putative major locus) correlation is not significantly different from zero ($p>0.05$) between these production traits and SaO but that the putative locus that affects SaO also affects Weight and Flesh in an overdominant fashion.

4.2.1.1 Power calculations

Power calculations were based on Weller *et al.* (1990). These authors provided an approximation of the power of half-sib designs (daughter and granddaughter-type designs), considering information of a single marker at a time in their calculations, which were based on the comparison of the marker allelic contrast to its standard error. Assumptions are that:

1. Sires are heterozygous at the marker locus, that is fully informative
2. Marker and QTL are completely linked
3. The QTL is biallelic –with alleles *B* and *b*-, is in Hardy-Weinberg equilibrium and has a negligible contribution the to total variance
4. The design is balanced with equal subgroup sizes at all levels (offspring/sire, offspring/sire/marker-allele)
5. Within-QTL variance known and similar to total variance.

Under these assumptions, power is a function of the total number of progeny and the proportion of variance explained by the QTL and can be derived by calculating the non-centrality parameter (λ) of a χ^2 distribution.

$$\lambda = [s\,(2\,p_B\,(1 - p_B))\,(a + d\,((1 - p_B) - p_B))^2] \,/\, [(1 - 0.25\,h_T)\,\sigma_p^2 \,/\, (n / 4)]$$

$$= s\,n\,\sigma_{QTL}^2 \,/\, (4 - h_T)$$

where s is the number of sires, p_B is the frequency of QTL allele *B*, a and d are the standardised QTL additive and dominance effects, h_T is the total heritability and σ_p^2 is the phenotypic variance and n is the number of offspring per sire. σ_{QTL}^2 is the variance explained by the QTL.

We used two different sets of parameters for the power calculations. Firstly, following the segregation analysis results obtained for ADJ(Data set 2) (see previous chapter), we assumed a QTL with $p_B = 0.65$ and $a = d = 0.87\,\sigma_p$ and $h_T = 0.40$. Secondly, we assumed QTL effect sizes $a = d = 0.40\,\sigma_p$ that corresponds roughly to half the size estimated in the segregation analysis and kept p_B and h_T as above.

4.2.1.2 Selection of sire families

Records on SaO, Weight and Flesh were taken at five weeks of age for all (male and female) progeny of sires selected from September 2001 and February 2002. 7dwt is routinely recorded within the breeding programme and was therefore readily available. Following the power study, we discarded all sire families with less than 50 offspring. The remaining sire families were ranked on the basis of the variance of the offspring Mendelian sampling terms (MST) for SaO.

4.2.1.3 Selection of offspring to genotype

Darvasi and Soller (1992) showed that, in most situations, it is not useful to genotype more than the upper and lower 25 % of the trait distribution when analysing marker-QTL linkage data with respect to a single trait, since most of the linkage information is contained in these data. Since our primary interest was on SaO, it was suggested to selectively genotype progeny for this trait within sire families.

60 samples can easily be accommodated on one gel run and for practical purposes it was decided to genotype samples from a given sire and 59 of his progeny (29 with the highest MST values and 30 with the lowest ones) in one gel run. Amongst the families with more than 50 offspring, 59 offspring corresponded to a proportion of between 42% and 84% of the total progeny. For the families selected to take part in the mapping study, it represented between 49% and 84% of the total offspring recorded.

4.2.2 Choice of candidate genes

The pathogenesis of the ascites syndrome is not fully understood. There is no agreement on what are its causes and what its effects amongst a variety of clinical signs observed in affected birds. Nonetheless, it is accepted that ascitic birds tend to be hypoxic and suffer from pulmonary hypertension and right ventricular hypertrophy, which can lead to heart failure. Recent studies in rats (Zhao *et al.*, 2001) have revealed the existence of a QTL linked to the right ventricular hypertrophy that occurs as a response to hypoxia-induced pulmonary hypertension, as observed in ascitic broilers. The RYR2 gene lies within this

QTL and has been implicated in the progression of ventricular myocytes dysfunction in hypertrophy and heart failure (Arai *et al.*, 1994). Studies in humans have also linked point mutations in the RYR2 gene to different cardiomyopathies (*e.g.* Tiso *et al.*, 2002).

RYR2 is one of three Ryanodine receptor isoforms identified in mammals. Ryanodine receptors (RYR) are a class of Ca^{2+}-selective ion channels located in the intracellular membrane systems of animal cells. These channels play a major role in the regulation of muscle excitation-contraction coupling (Sitsapesan and Williams, 1998). In mammals RYR1 and RYR2 are predominant isoforms in skeletal and cardiac muscle (although RYR2 is also expressed in brain tissue), whereas RYR3 is active in neurons and smooth muscle amongst others. Ottini *et al.* (1996) studied chicken RYR homologues and showed that in chickens, RYR1 is expressed in skeletal muscle (pectoral and thigh), testis and cerebellum, but not in heart, RYR2 can be detected in brain and cardiac muscle and RYR3 is expressed in pectoral and thigh muscles, as well as brain, testis and heart. These authors also showed that expression levels of the various isoforms in the tissues studied greatly differed from the patterns observed in mammals.

Based on this, the three chicken RYR homologues were chosen as candidate genes for our linkage study.

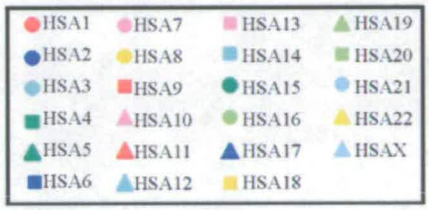
4.2.3 Choice of markers, genotyping and marker scoring

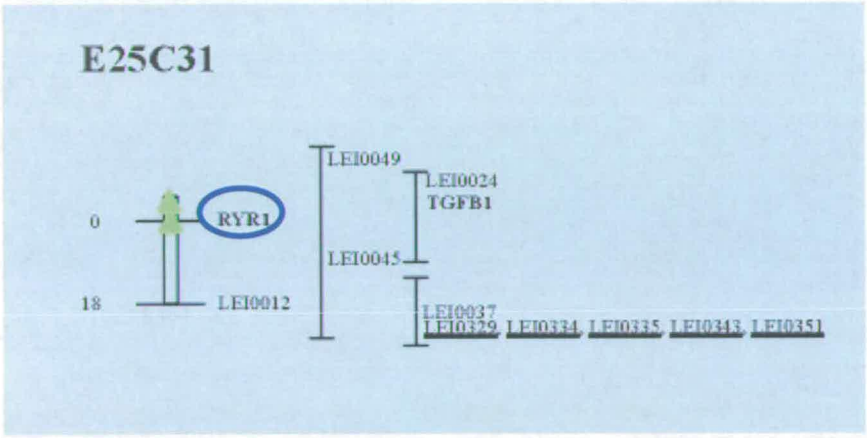
In chicken, RYR1 has been genetically mapped to location 0 cM of linkage group E25C31 (Groenen *et al.*, 2000). RYR2 is contained on the BAC bW015O09 that was isolated with microsatellite MCW0096 (Martien Groenen, personal communication). This microsatellite was genetically mapped to location 302 cM of chromosome 2 (GGA2). RYR3 was genetically mapped to location 95 cM of chromosome 5 (GGA5) (Smith *et al.*, 1997). Figure 4.1, adapted from http://www.zod.wau.nl/abg/hs/research/molecular/linkage_map.html shows the linkage groups harbouring the three RYR. Markers in the vicinity of the three RYR were found in Schmid *et al.* (2000).

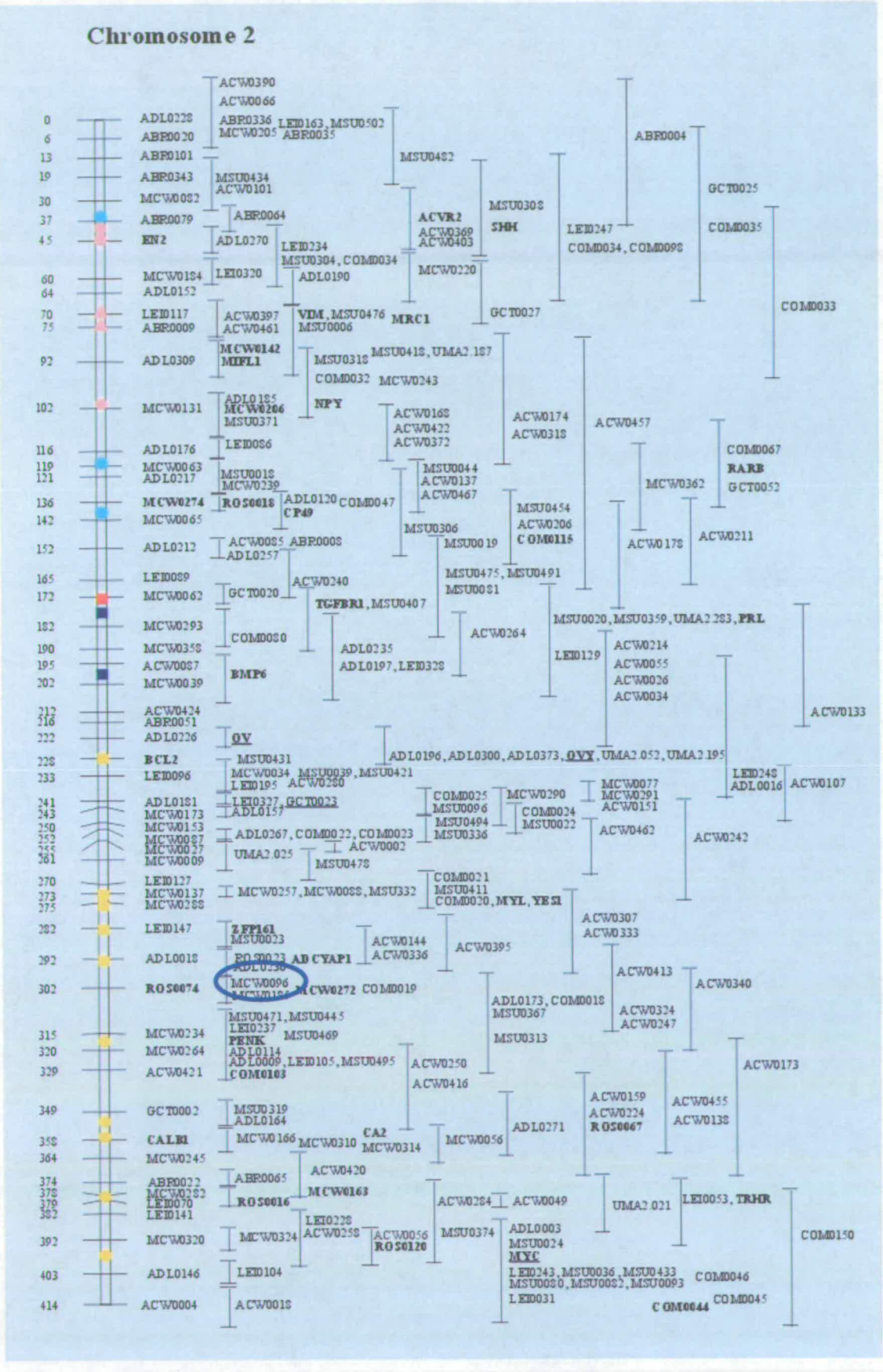
Figure 4.1. Linkage groups harbouring the three Ryanodine receptors. Estimated locations of Ryanodine receptors 1 and 3 (RYR1 and RYR3) are shown within blue ellipses; MCW0096 (also within a blue ellipse) is used to show the estimates location of Ryanodine receptor 2 (RYR2). The figure and the following legend are both from http://www.zod.wau.nl/abg/hs/research/molecular/linkage_map.html.

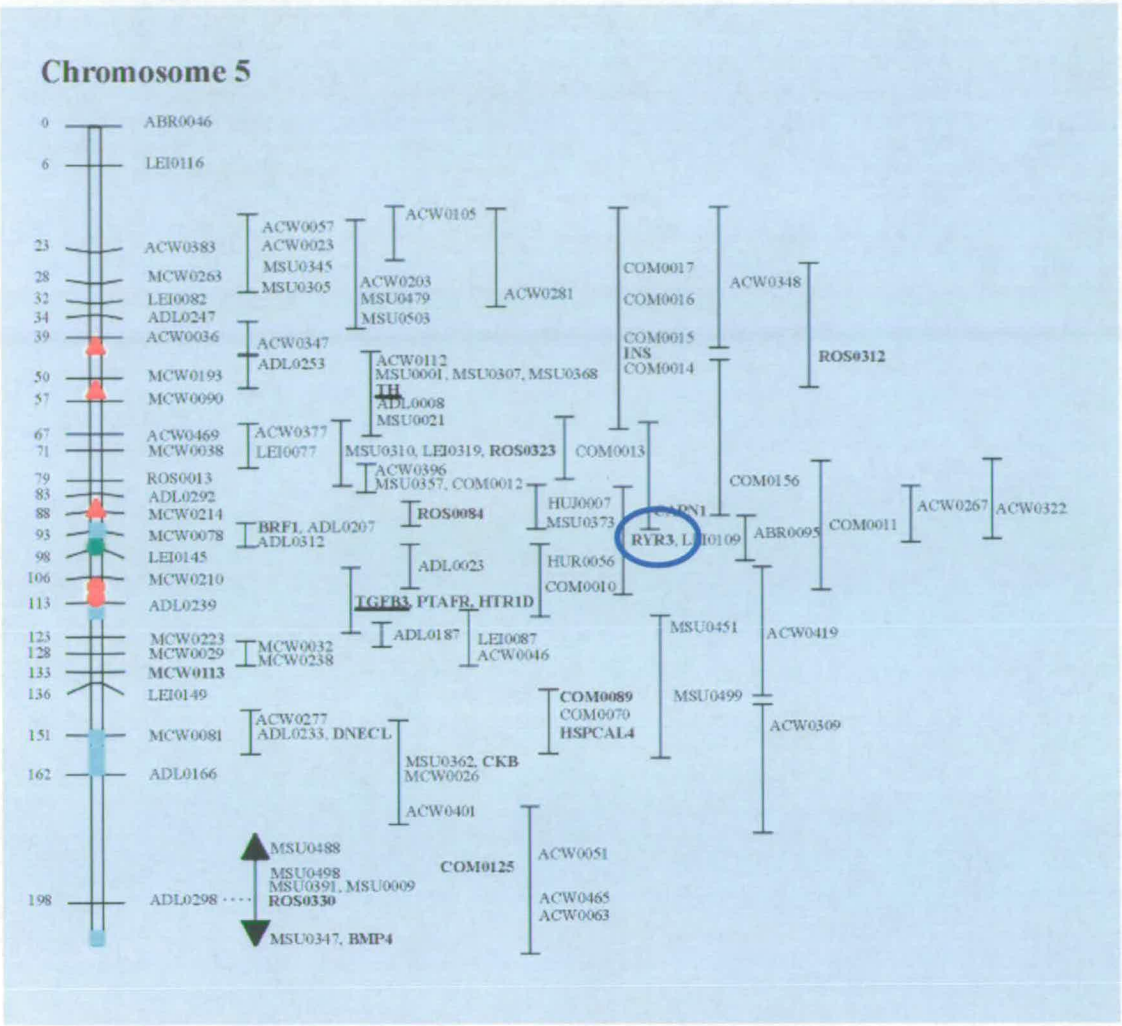
Framework loci (loci whose relative order is supported by odds larger than 3) have been ordered and they position is indicated by the number to the left. The possible location for the loci whose order is not supported by odds > 3 is indicated by an error bar. The loci that have been mapped cytogenetically are underlined. The loci known to represent expressed sequences (**identified genes** and **ESTs**) are in bold.

For genes whose map location on the human map is known, the human chromosome (HSAxx, where xx is the chromosome number) is indicated with a coloured symbol as show beside.



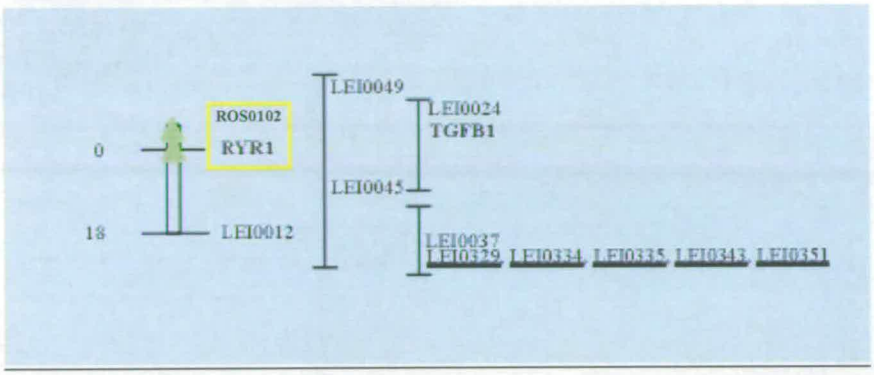


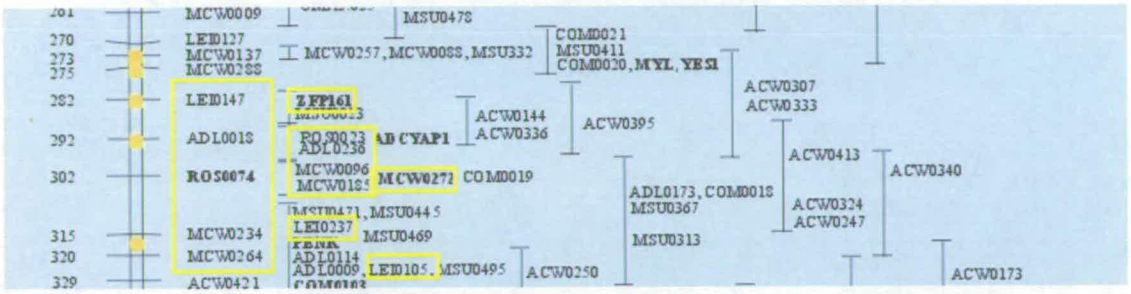

Chromosome 2
0
6
13
19
30
37
45
60
64
70
75
92
102
116
119
121
136
142
152
165
172
182
190
195
202
212
216
222
228
233
241
243
250
252
255
261
270
273
275
282
292
302
315
320
329
349
358
364
374
378
379
382
392
403
414
ADL0228
ABR0020
ABR0101
ABR0343
MCW0082
ABR0079
EN2
MCW0184
ADL0152
LEI0117
ABR0009
ADL0309
MCW0131
ADL0176
MCW0063
ADL0217
MCW0274
MCW0065
ADL0212
LEI0089
MCW0062
MCW0293
MCW0358
ACW0087
MCW0039
ACW0424
ABR0051
ADL0226
BCL2
LEI0096
ADL0181
MCW0173
MCW0153
MCW0087
MCW0027
MCW0009
LEI0127
MCW0137
MCW0288
LEI0147
ADL0018
ROS0074
MCW0234
MCW0264
ACW0421
GCT0002
CALR1
MCW0245
ABR0022
MCW0282
LEI0070
LEI0141
MCW0320
ADL0146
ACW0004
ACW0390
ACW0066
ABR0336
MCW0205
LEI0163, MSU0502
ABR0035
ABR0004
MSU0482
MSU0434
ACW0101
ABR0064
ADL0270
LEI0234
MSU0304, COM0034
LEI0320
ADL0190
MCW0220
ACVR2
ACW0369
ACW0403
SHH
MSU0308
LEI0247
COM0034, COM0098
GCT0025
COM0035
GCT0027
COM0033
MRC1
VIM, MSU0476
MSU0006
ACW0397
ACW0461
MCW0142
MIFL1
MSU0318
MSU0418, UMA2.187
COM0032
MCW0243
NPY
ADL0185
MCW0206
MSU0371
ACW0168
ACW0422
ACW0372
ACW0174
ACW0318
ACW0457
LEI0086
COM0067
RARB
GCT0052
MSU0044
ACW0137
ACW0467
MCW0362
MSU0018
MCW0239
ROS0018
ADL0120
CP49
COM0047
MSU0454
ACW0206
COM0115
MSU0306
MSU0019
ACW0178
ACW0211
ACW0085
ABR0008
ADL0257
ACW0240
GCT0020
TGFBR1, MSU0407
MSU0475, MSU0491
MSU0081
MSU0020, MSU0359, UMA2.283, PRL
COM0080
ACW0264
ADL0235
ADL0197, LEI0328
LEI0129
ACW0214
ACW0055
ACW0026
ACW0034
ACW0133
BMP6
OV
ADL0196, ADL0300, ADL0373, OVY, UMA2.052, UMA2.195
MSU0431
MCW0034, MSU0039, MSU0421
LEI0195
ACW0280
LEI0248
ADL0016
ACW0107
LEI0327, GCT0023
ADL0157
COM0025
MSU0096
MCW0290
MCW0077
MCW0291
ACW0151
COM0024
MSU0022
MSU0494
MSU0336
ACW0462
ACW0242
ADL0267, COM0022, COM0023
ACW0002
UMA2.025
MSU0478
COM0021
MSU0411
COM0020, MYL, YES1
MCW0257, MCW0088, MSU332
ZFP161
MSU0023
ACW0144
ACW0336
ACW0395
ACW0307
ACW0333
ADCYAP1
ROS0023
ACW0413
ACW0340
MCW0096
MCW0272
COM0019
ADL0173, COM0018
MSU0367
ACW0324
ACW0247
MSU0471, MSU0445
LEI0237
PENK
MSU0469
ADL0114
ADL0009, LEI0105, MSU0495
COM0103
MSU0313
ACW0250
ACW0416
ACW0173
MSU0319
ADL0164
ACW0159
ACW0224
ROS0067
ACW0455
ACW0138
CA2
MCW0166
MCW0310
MCW0314
MCW0056
ADL0271
ACW0420
MCW0163
ABR0065
ROS0016
ACW0284
ACW0049
UMA2.021
LEI0053, TRHR
LEI0228
ACW0258
ACW0056
ROS0120
MSU0374
ADL0003
MSU0024
MYC
COM0150
LEI0104
LEI0243, MSU0036, MSU0433
MSU0080, MSU0082, MSU0093
COM0046
LEI0031
COM0044
COM0045
ACW0018

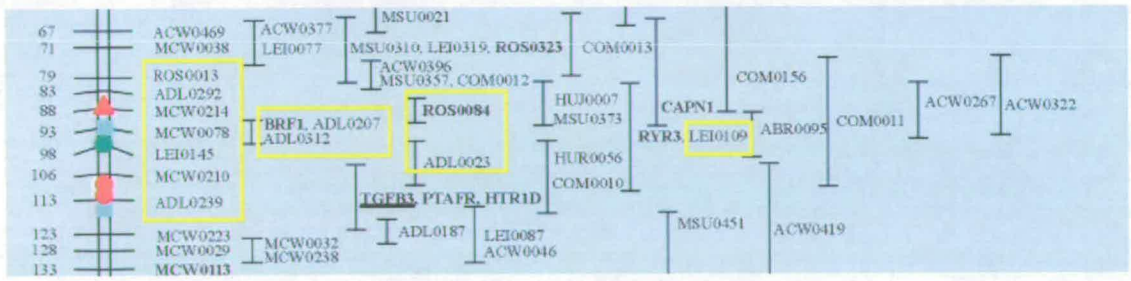

Chromosome 5
0 ABR0046
6 LEI0116
23 ACW0383
28 MCW0263
32 LEI0082
34 ADL0247
39 ACW0036
50 MCW0193
57 MCW0090
67 ACW0469
71 MCW0038
79 ROS0013
83 ADL0292
88 MCW0214
93 MCW0078
98 LEI0145
106 MCW0210
113 ADL0239
123 MCW0223
128 MCW0029
133 MCW0113
136 LEI0149
151 MCW0081
162 ADL0166
198 ADL0298
ACW0057
ACW0023
MSU0345
MSU0305
ACW0105
ACW0203
MSU0479
MSU0503
ACW0281
ACW0347
ADL0253
ACW0112
MSU0001, MSU0307, MSU0368
IH
ADL0008
MSU0021
ACW0377
LEI0077
MSU0310, LEI0319, ROS0323
ACW0396
MSU0357, COM0012
COM0013
COM0017
COM0016
COM0015
INS
COM0014
ACW0348
ROS0312
COM0156
HUJ0007
MSU0373
CAPN1
RYR3, L
I0109
ABR0095
COM0011
ACW0267
ACW0322
ROS0084
BRF1, ADL0207
ADL0312
ADL0023
TGFB3, PTAFR, HTR1D
HUR0056
COM0010
ADL0187
LEI0087
ACW0046
MCW0032
MCW0238
MSU0451
ACW0419
COM0089
COM0070
HSPCAL4
MSU0499
ACW0277
ADL0233, DNECL
MSU0362, CKB
MCW0026
ACW0401
ACW0309
MSU0488
MSU0498
MSU0391, MSU0009
ROS0330
MSU0347, BMP4
COM0125
ACW0051
ACW0465
ACW0063

Given the technology available, choice of markers was restricted to PCR-based markers. Microsatellite markers were preferred to less polymorphic markers and we focused on microsatellite markers around locations of RYR2 (13 markers, covering around 50 cM in the consensus linkage map) and RYR3 (13 markers, covering around 40 cM in the consensus linkage map). For the RYR1 region a PCR-RFLP marker within the RYR1 gene was considered. Figure 4.2 shows a subset of the available markers that was chosen and tested on the twenty sires from the mapping population. Details on the markers tested were found at http://www.thearkdb.org/browser and http://flex026.zod2.wau.nl/cgi-bin/ace/grep/chickace. All genotyping was carried out at Aviagen Ltd. laboratories by Stewart Brown and no details on PCR conditions or gel electrophoresis will be provided here. Marker polymorphism, sire heterozygosity, location and technical quality of gels determined the final set of markers to be used in the mapping study.

Figure 4.2. Subset of the available markers that was chosen and tested on the twenty sires from the mapping population around the three Ryanodine receptor regions (shown within yellow rectangles).







Once these markers were chosen, gels were independently scored by Stewart Brown and Pau Navarro, scores were compared and discrepancies resolved by setting individual marker scores to unknown for ten birds. After agreement on gel scores, dam genotypes were inferred from the genotypes of mate and progeny when possible and used in further analyses. Potential genotyping or pedigree errors were identified from genotype scorings. Mainly, problems were due to the existence of too many possible alleles within full-sib families, implying that the ungenotyped dam had more than two alleles. This problem arose for six full-sib families (53 offspring in total) for one or more markers in the RYR2 linkage group and six full-sib families (41 offspring in total) for one or more markers in the RYR3 linkage group. All marker genotypes of birds belonging to these families were set to unknown. Additionally, three offspring did not have any of their sire alleles for one or more markers in RYR2 or RYR3 linkage groups. In total genotypes of 87 birds were set to unknown.

4.2.4 Map construction

The linkage map was constructed from the study data using Cri-map (Green *et al.*, 1990). The final map order was checked using the "flipsn" option from this package.

4.2.5 QTL analyses and significance thresholds

QTL analyses were conducted using a least squares framework, following the multiple-marker method developed by Knott *et al.* (1996) for half-sib populations. The web-based software package QTL Express (Seaton *et al.*, 2002) implements this method and was used to perform the QTL analyses.

The analysis proceeds in two steps. Firstly, the probabilities of each half-sib of inheriting a given allele from their sire (p_l, "coefficients") are calculated using marker data on progeny and sire in a multi-point approach. These probabilities can be used to calculate marker information content and segregation distortion. Secondly, offspring phenotypes are regressed onto the probabilities of inheriting a given allele from their sire for every cM. The regression is performed within half-sib family, making no assumptions on the number of alleles at the QTL. The test statistic is a standard F-test resulting from the comparison of a

model with a QTL with a model with no QTL and the most likely QTL position is the one with the highest test statistic for each linkage group. Point-wise and chromosome-wide significance were assessed using permutation analysis as implemented in QTL Express. 1000 permutations were used in the permutation analyses. For each half-sib family, a t-statistic is calculated at the most likely QTL position (using the estimate of the sire allele substitution effect and its standard error) and can be used to infer which sire families are likely to be segregating at the QTL once evidence of segregation has been found using across-family information. Individual sire t-statistics were compared to tabulated values.

In a first stage, each linkage group was searched for a single QTL assuming sex-equal effects. Because ascites incidence is greater in males than in females, we fitted a model that allowed the QTL to have different effects across sexes at every marker location. These analyses were done with the Fit directive from GENSTAT (GENSTAT 5 COMMITTEE, 1993) using the coefficients obtained from QTL Express for these locations. The model including a sex interaction was compared with a model without QTL and a model without interaction using an F ratio. Point-wise thresholds obtained from permutation analyses for models with sex-equal effects were approximately the ones that would be obtained from a standard F distribution table (with the number of sires being the numerator degrees of freedom and the residual degrees of freedom of a model with a QTL being the denominator degrees of freedom) for all traits, therefore tabulated values were used as point-wise thresholds for analyses including a sex interaction, (the numerator degrees of freedom are in this case twice the number of sires). QTL models with and without sex interaction were compared using the nominal point-wise significance.

4.3 Results

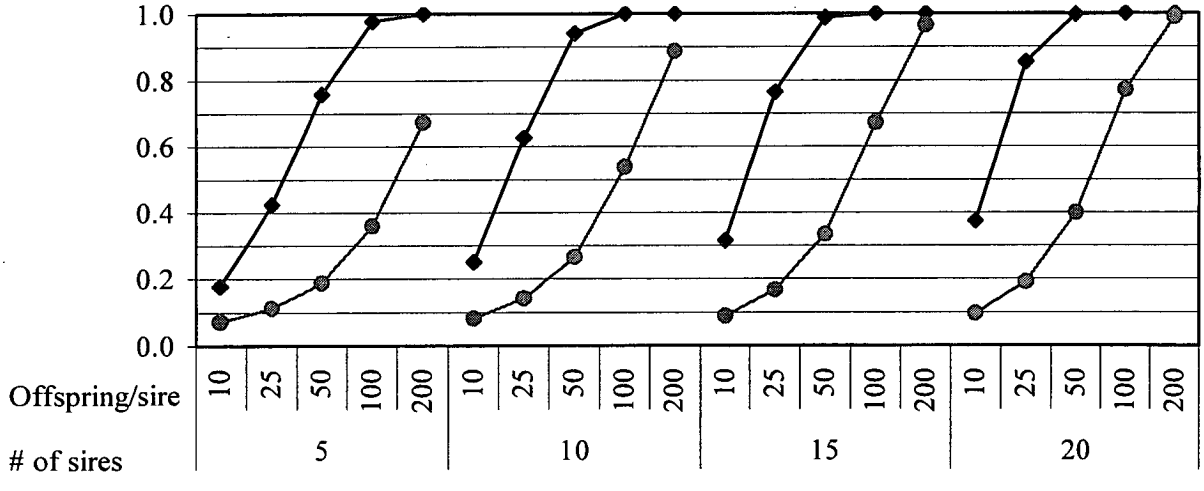
4.3.1 Experimental design: Power study, mapping population and traits

4.3.1.1 Power calculations

Figure 4.3 shows the power to detect a QTL (5% significance level) as a function of its standardised additive and dominance effects (a and d), for $a = d = 0.87\ \sigma_P$ and $a = d =$

0.40 σ_P, the number of sires (5, 10, 15 or 20) and offspring per sire (10, 25, 50, 100 or 200) for a half-sib design, assuming a total heritability of 0.40 and a bi-allelic QTL with a frequency of 0.65 of the increasing allele. Collecting data from 20 sire families with 50 or more offspring per family would allow to detect a QTL with size and allele frequency as estimated from the segregation analysis of SaO saturation data with a power greater than 0.99. For the same number of sire families and a QTL approximately half the size of the one suggested by previous studies, the power of the design is around 0.40 for a family size of 50 and close to 0.80 for families of 100 half-sibs. For an effect size of 0.87 σ_P and a population of 20 sires with 50 offspring, the power to detect a QTL is respectively 0.91 and 0.83 when the type I error rate is set to 0.0005 and 0.0001.

Figure 4.3. Power to detect a QTL (5% significance level) as a function of its standardised additive and dominance effects (*a* and *d*, $a = d = 0.87\ \sigma_P$ is shown as black diamonds ♦ and $a = d = 0.40\ \sigma_P$ as grey dots •) and the number of sires (5, 10, 15 or 20) and offspring per sire (10, 25, 50, 100 or 200) for a half-sib design, assuming a total heritability of 0.40 and a bi-allelic QTL with a frequency of 0.65 of the increasing allele.



Power calculations as performed provide only a rough approximation of power, since in practice, none of the assumptions made are fulfilled, which results in too optimistic figures. It was decided therefore to collect data on at least 20 sire families and record all

available offspring in these families, with the aim of obtaining as close to 100 offspring per family. In the population studied, within the available breeding population structure, mean paternal half-sib family size was around 50 with maximum values around 130. Aiming to record as many progeny per sire family as possible should allow families of more than 50 progeny to be obtained.

At the end of the recording period, after discarding families with less than 50 progeny, 38 families were available. A segregation analysis carried out in this population (referred to as Data set 3 in chapter three) yielded estimates of genotype probabilities for the 38 sires, and 34 out of the 38 had an estimated probability greater than 0.99 of being heterozygous at the putative major locus, the remaining 4 having a high (>0.80) probability of being homozygous with high trait values. Families were ranked on the basis of progeny MST variance, and the 20 families with highest variance were chosen as the mapping population. This selection left out the four sire families for which the sire had a low probability of being heterozygous at a putative major locus as estimated from the segregation analysis. In total, 20 sires and 59 offspring from each sire (totalling 172 full-sib families) were selected to be genotyped. Table 4.1 shows mean, minimum and maximum values and residual standard deviations (after adjustment for effects of half and full-sib family, sex and hatch) of all traits analysed for the 1180 mapping progeny.

Table 4.1. Mean, minimum, maximum values and residual standard deviations (σ_p) of all traits analysed for the 1180 mapping progeny. Trait units are shown in brackets.

Trait	Mean	Minimum	Maximum	σ_p
SaO (%)	79.96	39.00	98.00	10.96
Ln(100-SaO) (units)	2.83	0.69	4.11	0.60
Flesh (units)	3.10	1.00	5.00	0.89
Weight (dag)	197.70	132.00	256.00	15.00
Selwt (dag)	197.30	118.00	256.00	17.59
7dwt (g)	180.30	88.00	253.00	15.14
Gain (g)	1798.00	1148.00	2380.00	144.64

4.3.2 *Choice of markers and genotyping*

After testing markers in the 20 sires of the half-sib families selected for the study, the markers on the RYR2 and RYR3 regions to be used in the mapping offspring were selected on the basis of informativeness, location (so as to cover the interval of the possible location of MCW0096 and RYR3 on the consensus map (Schmid *et al.*, 2000) respectively for RYR2 and RYR3) and ease of scoring. The set of chosen markers was:

RYR1 linkage group: ROS0102, located within the RYR1 gene

RYR2 linkage group: ADL0236, ROS0023 and LEI0237

RYR3 linkage group: ROS0013, ADL0292, ADL0312, ROS0084, ADL0023 and MCW0210

Not all markers were informative for all sires (see Table 4.2). Within each sire family, progeny were only genotyped for informative markers.

4.3.3 *Genetic map*

Locus order and sex-averaged genetic distances were obtained from the study data for markers on RYR2 and RYR3 linkage groups. The relative order of ADL0236 and LEI0237 and ROS0013, ADL0292, ROS0084 and ADL0023 respectively was supported by odds larger than 3 and was consistent with orders and distances from the consensus linkage map. The location of ADL0312 on the RYR3 linkage group could not be determined unequivocally since two linkage group configurations yielded equal likelihoods. The configuration chosen for further analyses was the one closest to the consensus map. The maps used for the QTL analyses are:

RYR2 linkage group: ROS0023 1.01 cM ADL0236 19.28 cM LEI0237. The total estimated length for this linkage group was about 20 cM and the average distance between markers around 10 cM.

RYR3 linkage group: ROS0013 3.09 cM ADL0292 3.09 cM ROS0084 8.71 cM ADL0312 2.04 cM ADL0023 0.00cM MCW0210. The total estimated length for this linkage group was about 17 cM and the average distance between markers around 3.5 cM.

4.3.4 *Marker information summary*

Table 4.2, adapted from QTL Express, shows a summary of the information available at marker locations for the three RYR regions studied. On average at every marker location about 10 sires were informative, only 37 dam genotypes could be inferred and around 645 offspring were ungenotyped or have had their genotypes set to unknown due to inconsistent marker data. The average number of alleles per marker was 4.5.

Table 4.2. Number of genotyped (i.e., heterozygous, here) sires, dams without inferred genotype, ungenotyped progeny and alleles at marker locations for the three RYR regions studied. Total number of sires, dams and progeny are show in brackets.

Region	RYR1	RYR2			RYR3					
Marker	ROS0102	ROS0023	ADL0236	LEI0237	ROS0013	ADL0292	ROS0084	ADL0312	ADL0023	MCW0210
# Heterozygous Sires (20)	6	15	16	15	6	17	16	1	3	4
# Dams without inferred genotype (172)	172	109	118	123	135	105	94	169	163	162
# Ungenotyped progeny (1180)	821	366	327	371	848	258	361	1126	1029	948
# Alleles	2	4	5	6	6	5	7	3	4	3

Marker information content for RYR1 was around 0.12 when taking all half-sib families into account and around 0.40 when calculating information content using only segregating families. Figures 4.4 and 4.5 show information content along RYR2 and RYR3 linkage groups respectively. For RYR2 linkage group, information content varied from 0.60 to 0.71, with a mean value of 0.64. For RYR3 linkage group, minimum, maximum and mean information content were respectively 0.65, 0.84 and 0.76. Table 4.3 shows the number of uninformative and informative progeny per half-sib family. The number of informative progeny was estimated as the number of progeny with at least one informative marker per linkage group.

Figure 4.4. Marker information content (IC) along RYR2 linkage group. Marker locations are shown with red triangles (▲).

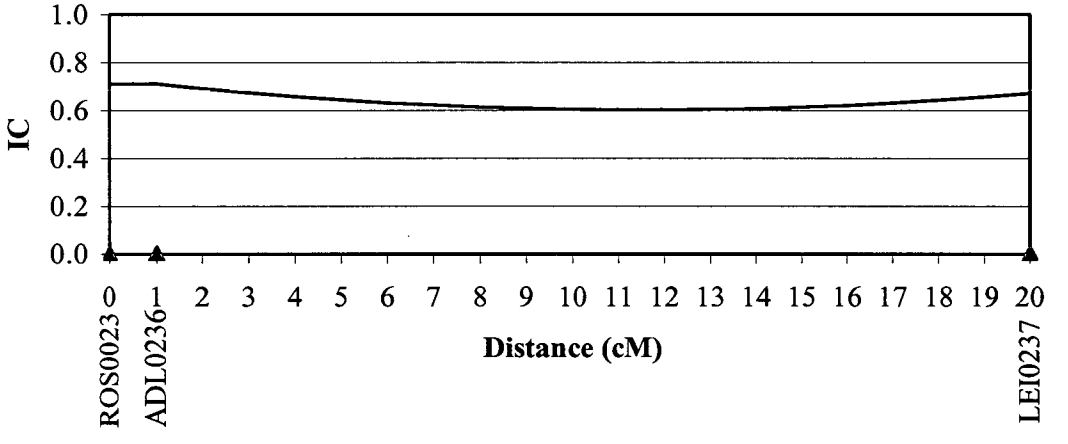


Figure 4.5. Marker information content (IC) along RYR3 linkage group. Marker locations are shown with red triangles (▲).

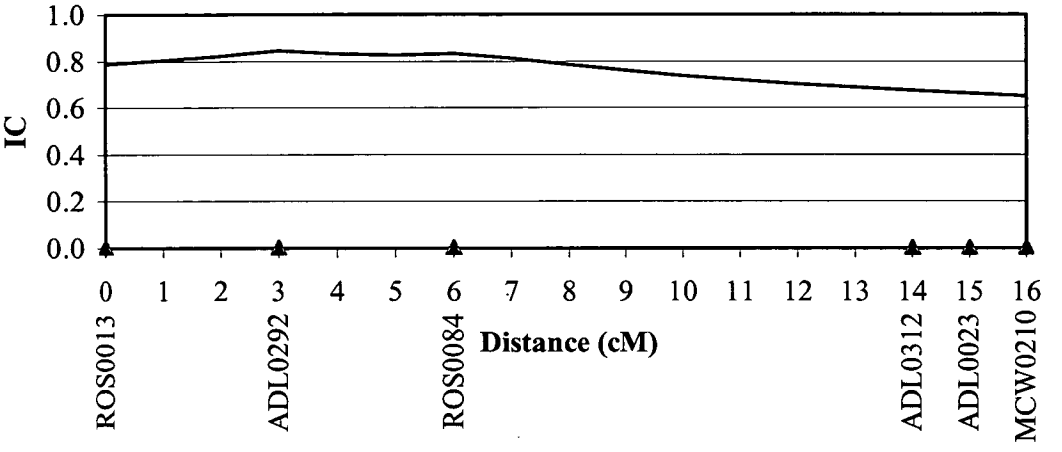


Table 4.3. Number of uninformative and informative progeny per half-sib family for each Ryanodine receptor linkage group (RYR1, RYR2 and RYR3). The number of informative progeny was estimated as the number of progeny with at least one informative marker per linkage group.

	Sire	569	574	579	580	581	588	592	594	602	605	610	612	616	618	619	620	622	624	626	627
RYR1	U [total=1029]	59	59	59	59	59	59	59	33	59	59	59	59	41	59	59	38	26	23	42	59
	I [total=151]	0	0	0	0	0	0	0	26	0	0	0	0	18	0	0	21	33	36	17	0
RYR2	U [total=197]	5	5	4	5	3	8	6	15	37	2	2	7	10	0	10	18	17	4	28	11
	I [total=983]	54	54	55	54	56	51	53	44	22	57	57	52	49	59	49	41	42	55	31	48
RYR3	U [total=139]	1	2	4	3	2	2	5	14	27	3	0	1	9	4	13	8	2	4	25	10
	I [total=1041]	58	57	55	56	57	57	54	45	32	56	59	58	50	55	46	51	57	55	34	49

4.3.5 *QTL analyses*

Tables and figures presented in this section have been adapted from QTL Express. Table 4.4 shows the location with the highest test statistic, together with an indication of point-wise significance for all traits and linkage groups. For locations with point-wise significance greater than 10% an indication of chromosome-wide significance is given, together with the percentage of the variance accounted for by the QTL. No confidence intervals for QTL locations are presented.

Table 4.4. Location with the highest test statistic (cM) and highest test statistic (F) for all traits and linkage groups (LG). An indication of point-wise significance (Ppoint) is also given and, for locations with point-wise significance greater than 10%, an indication of and chromosome-wide significance (Pchrom) and the percentage of the residual variance accounted for by the QTL (%) are presented.

LG	TRAIT	cM	F	Ppoint	Pchrom	%
RYR1	SaO	0	1.07	0.38		
	Ln(100-SaO)	0	1.20	0.32		
	Flesh	0	0.70	0.65		
	Weight	0	0.52	0.77		
	Selwt	0	0.57	0.76		
	7dwt	0	0.20	0.97		
	Gain	0	0.61	0.72		
RYR2	SaO	0	0.78	0.76		
	Ln(100-SaO)	20	0.71	0.81		
	Flesh	20	0.52	0.96		
	Weight	20	0.90	0.60		
	Selwt	20	0.92	0.58		
	7dwt	20	1.14	0.35		
	Gain	20	0.93	0.52		
RYR3	SaO	6	1.00	0.46		
	Ln(100-SaO)	6	1.17	0.28		
	Flesh	5	2.39	0.00	<0.001	2.75
	Weight	0	1.56	0.06	0.07	1.13
	Selwt	0	1.61	0.04	0.07	1.24
	7dwt	0	0.87	0.64		
	Gain	0	1.59	0.05	0.07	1.19

No evidence of QTL segregation was found on RYR1 or RYR2 linkage groups for any of the traits studied. A QTL for fleshing score, significant at 0.1% chromosomal level, was identified at position 5 cM on RYR3 linkage group, and indication of QTL segregating for Selwt, Weight and Gain was also found at position 0 cM (between 0.04 and 0.06 point-wise significance). Figures 4.6 and 4.7 show test statistic profiles for RYR2 and RYR3 linkage groups obtained for all traits.

Figure 4.6. F ratio profiles obtained for blood oxygen saturation (raw and transformed data; SaO and Ln(100-SaO)), fleshing score (Flesh) and body weight (Weight) at five weeks of age, selection weight (Selwt), body weight at seven days of age (7dwt) and weight gain between seven days and five weeks (Gain) for the region of chromosome 2 harbouring Ryanodine receptor 2 (RYR2). Marker locations are marked with red triangles (▲).

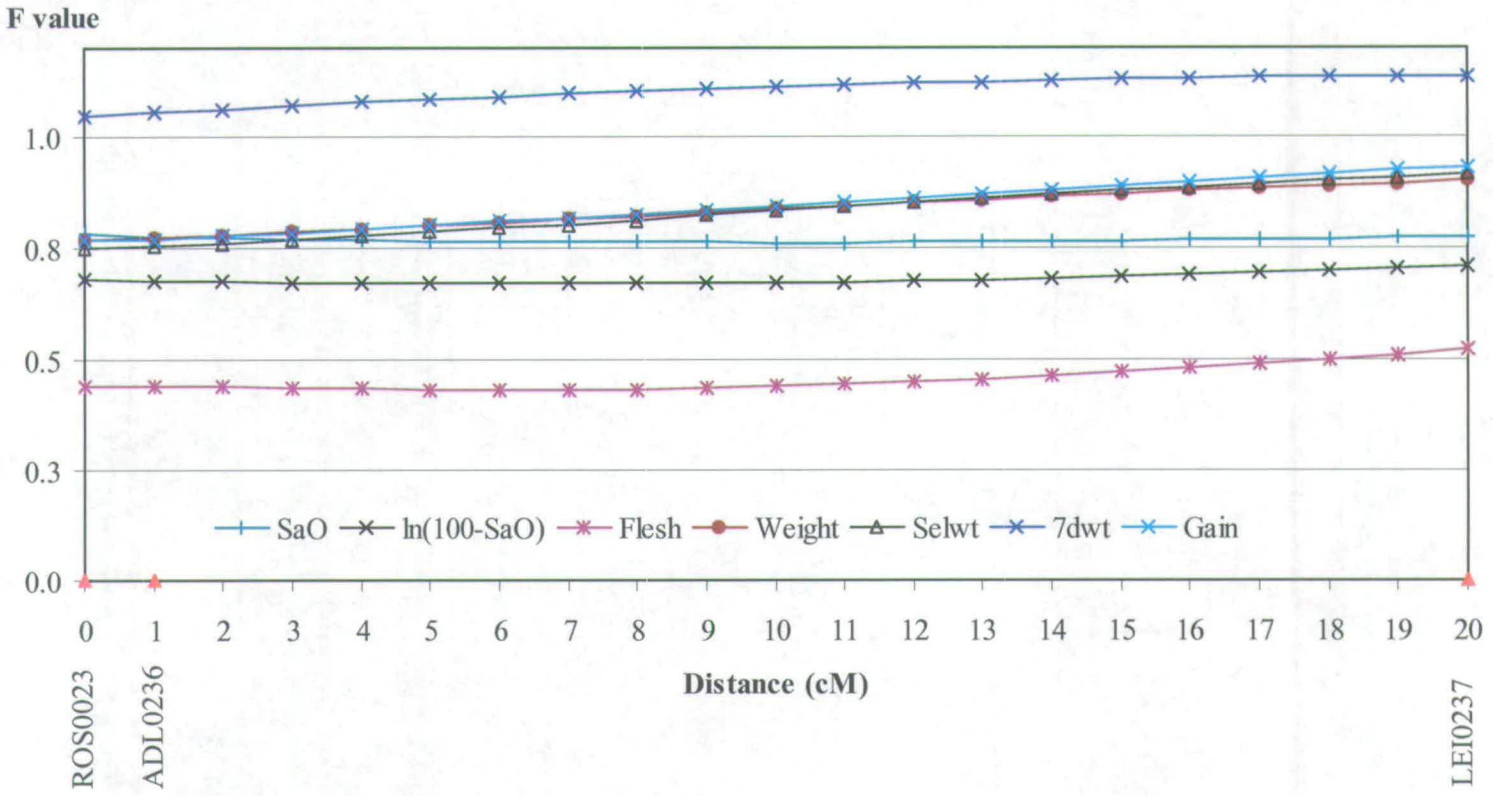


Figure 4.7. F ratio profiles obtained for blood oxygen saturation (raw and transformed data; SaO and Ln(100-SaO)), fleshing score (Flesh) and body weight (Weight) at five weeks of age, selection weight (Selwt), body weight at seven days of age (7dwt) and weight gain between seven days and five weeks (Gain) for the region of chromosome 5 harbouring Ryanodine receptor 3 (RYR3). Marker locations are marked with red triangles (▲). Approximate 5% point-wise and 1% chromosome-wide thresholds are shown in red dashes (-) and crosses (+) respectively.

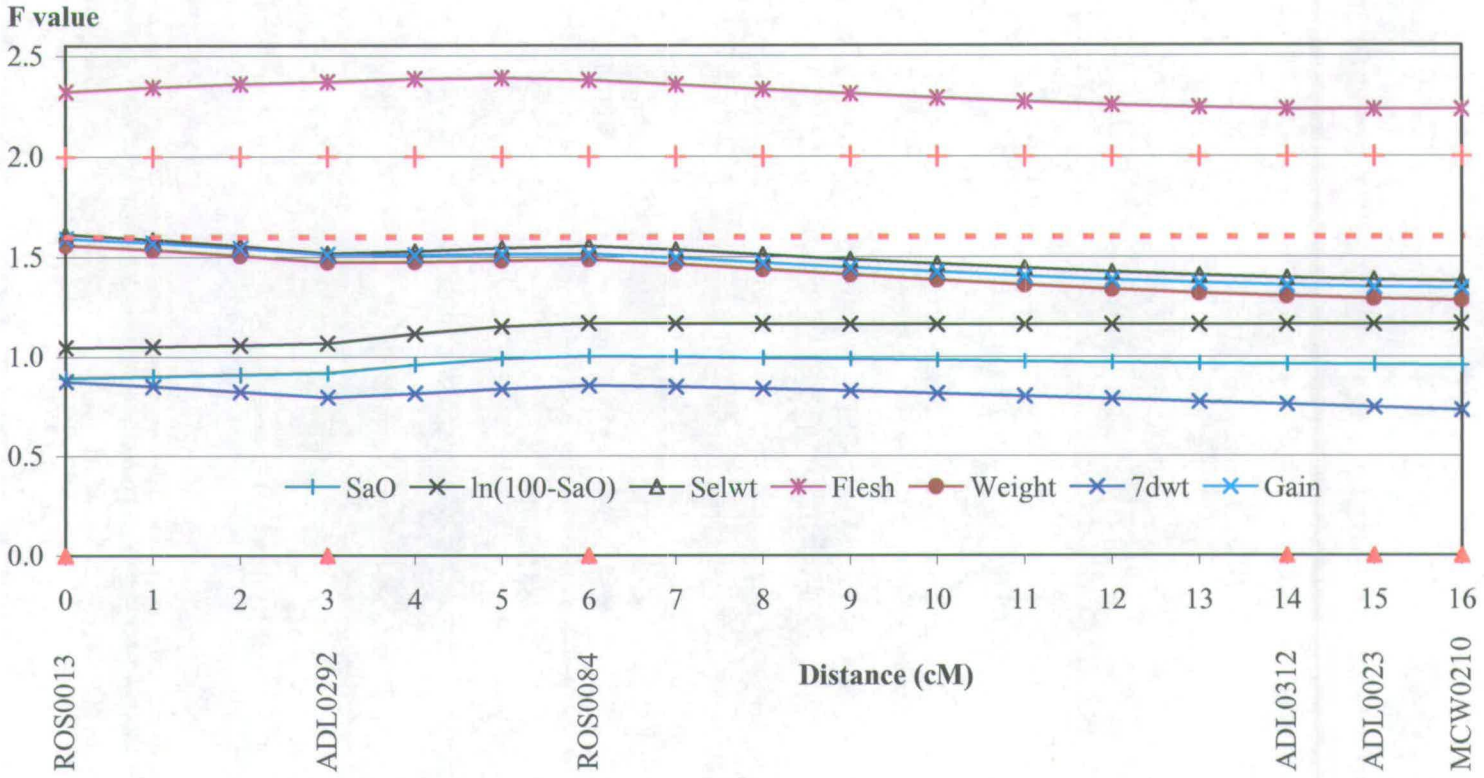


Table 4.5 presents estimates of the allele substitution effect for each sire (in absolute units and in residual standard deviation units) for the QTL for fleshing score found on RYR3 linkage group, together with standard errors of the estimates and an indication of the significance of the contrast. Sires are shown in decreasing order of absolute t-statistics. Estimates of allele substitution effect were obtained at the location with the highest F-statistic for this trait. The sign of the allele substitution effect is arbitrary. For the four sires that showed evidence ($p<0.05$) of segregation for the putative fleshing score QTL, absolute standardised effects ranged from 0.66 to 1.37. Table 4.6 shows the same parameters (estimated at position 0 cM) for QTL segregating for Selwt, Weight and Gain found the same linkage group. For all traits, two sires were segregating ($p<0.05$) at the QTL and standardised QTL effects for these sires were about 0.70. Sire 588 ($p<0.05$) was inferred to be segregating for both the weight and the breast conformation QTL.

Table 4.5. Estimates of allele substitution effects for each sire, in absolute units (α) and in residual standard deviation units ($SD\alpha$) for the fleshing score QTL on RYR3 linkage group. The standard error of the estimate (SE), the corresponding absolute t-statistic ($|t|$) and an indication of the significance of the contrast (P) are also presented.

Sire	α	SE	$SD\alpha$	$\|t\|$	P
626	1.23	0.32	1.37	3.78	0.00
588	0.76	0.26	0.85	2.96	0.00
610	-0.59	0.26	-0.66	2.31	0.02
620	0.62	0.28	0.69	2.21	0.03
624	-0.49	0.25	-0.55	1.95	0.05
574	-0.46	0.26	-0.52	1.81	0.07
592	0.35	0.27	0.39	1.29	0.20
594	-0.36	0.29	-0.40	1.22	0.22
569	0.24	0.25	0.27	0.96	0.34
618	-0.23	0.26	-0.26	0.88	0.38
616	0.26	0.30	0.29	0.86	0.39
581	0.22	0.28	0.25	0.79	0.43
627	-0.22	0.29	-0.25	0.77	0.44
622	-0.14	0.28	-0.15	0.49	0.63
602	-0.16	0.34	-0.18	0.47	0.64
612	0.11	0.26	0.13	0.44	0.66
580	-0.05	0.25	-0.06	0.20	0.84
605	-0.04	0.26	-0.04	0.14	0.89
619	0.04	0.30	0.05	0.13	0.89
579	-0.03	0.25	-0.03	0.11	0.92

Table 4.6. Estimates of allele substitution effects for each sire, in absolute units (α) and in residual standard deviation units (SDα) for the QTL for weight at five weeks (Weight), selection weight (Selwt) and weight gain between seven days and five weeks of age (Gain) on RYR3 linkage group. The standard error of the estimate (SE) the corresponding absolute t-statistic (|t|) and an indication of the significance of the contrast (P) are also presented.

	Weight					Selwt					Gain				
Sire	α	SE	SDα	|t|	P	α	SE	SDα	|t|	P	α	SE	SDα	|t|	P
588	11.24	4.31	0.75	2.61	0.01	12.02	5.05	0.68	2.38	0.02	114.70	41.52	0.79	2.76	0.01
627	-10.15	5.02	-0.68	2.02	0.04	-12.39	5.88	-0.70	2.11	0.04	-105.40	48.37	-0.73	2.18	0.03
626	10.56	5.88	0.70	1.80	0.07	13.50	6.90	0.77	1.96	0.05	105.18	56.71	0.73	1.85	0.06
610	-6.67	4.22	-0.44	1.58	0.11	-7.75	4.95	-0.44	1.57	0.12	-62.27	40.72	-0.43	1.53	0.13
574	-6.92	4.54	-0.46	1.52	0.13	-7.54	5.33	-0.43	1.41	0.16	-62.76	43.81	-0.43	1.43	0.15
602	-9.42	6.44	-0.63	1.46	0.14	-10.37	7.55	-0.59	1.37	0.17	-87.36	62.09	-0.60	1.41	0.16
620	6.35	4.48	0.42	1.42	0.16	8.07	5.25	0.46	1.54	0.12	55.11	43.19	0.38	1.28	0.20
624	-4.98	4.46	-0.33	1.12	0.26	-6.43	5.22	-0.37	1.23	0.22	-43.76	42.97	-0.30	1.02	0.31
581	-5.08	4.99	-0.34	1.02	0.31	-6.62	5.85	-0.38	1.13	0.26	-44.81	48.14	-0.31	0.93	0.35
612	-4.72	4.65	-0.31	1.01	0.31	-4.87	5.46	-0.28	0.89	0.37	-51.10	44.87	-0.35	1.14	0.26
592	4.66	4.74	0.31	0.98	0.33	6.06	5.56	0.34	1.09	0.28	49.30	45.71	0.34	1.08	0.28
605	4.19	4.28	0.28	0.98	0.33	5.16	5.02	0.29	1.03	0.30	38.30	41.30	0.26	0.93	0.35
579	-4.15	4.50	-0.28	0.92	0.36	-5.39	5.28	-0.31	1.02	0.31	-40.96	43.40	-0.28	0.94	0.35
619	-4.93	5.49	-0.33	0.90	0.37	-8.27	6.44	-0.47	1.28	0.20	-49.08	52.95	-0.34	0.93	0.35
622	-3.10	4.49	-0.21	0.69	0.49	-3.06	5.26	-0.17	0.58	0.56	-24.28	43.25	-0.17	0.56	0.57
618	-3.02	4.65	-0.20	0.65	0.52	-3.49	5.46	-0.20	0.64	0.52	-28.50	44.87	-0.20	0.64	0.53
616	2.76	5.46	0.18	0.51	0.61	3.67	6.40	0.21	0.57	0.57	22.80	52.63	0.16	0.43	0.66
569	1.16	4.51	0.08	0.26	0.80	1.00	5.28	0.06	0.19	0.85	12.03	43.45	0.08	0.28	0.78
580	0.79	4.42	0.05	0.18	0.86	1.01	5.18	0.06	0.19	0.85	11.91	42.58	0.08	0.28	0.78
594	0.64	5.24	0.04	0.12	0.90	1.24	6.14	0.07	0.20	0.84	9.80	50.49	0.07	0.19	0.85

Estimates of effects for all traits were obtained at the location of the putative QTL for fleshing score and the correlation of standardised effects calculated. Standard errors of the effect estimates varied between sires but variation was small. Rank correlations of the sires absolute t-statistics were also obtained. Tables 4.7 and 4.8 show correlations of standardised effects between traits and rank correlations of absolute t-statistics. Correlations of effects can be seen as genetic correlations at the QTL (that are a composite of correlations due to this and linked QTL and polygenic correlation) and rank correlations of absolute t-statistics indicate if the sires that are likely to segregate for a QTL for one trait are likely to be segregating for a QTL for another trait. The correlation of effect estimates between SaO and Ln(100-SaO) was close to –1 as expected because of the nature of the transformation. Correlations amongst these and production traits were not different from zero ($p>0.05$), which was also the estimated correlation from multitrait animal model analyses. Correlations of effects between Flesh and Weight, Selwt and Gain were positive and high (close to 0.80) which was slightly greater than the correlation of 0.62 (0.02) estimated between Flesh and Weight from multitrait animal model analyses. The genetic correlations between Flesh and Weight and 7dwt estimated using an animal model were 0.28 and 0.60 (A. Koerhuis, personal communication) and these are respectively slightly higher and slightly lower than the correlation of estimated effects. Since Weight, Selwt and Gain are highly related traits, the estimated correlation close to or equal to unity amongst these traits meets our expectations.

Table 4.7. Correlation of sire estimated QTL standardised effects at the position of the putative fleshing score QTL on Ryanodine receptor 3 linkage group between traits. P-values for the hypothesis test of the correlation coefficient being zero are shown in brackets below estimated correlations.

	SaO	Ln(100-SaO)	Flesh	Weight	Selwt	7dwt
Ln(100-SaO)	-0.96 (0.00)					
Flesh	0.17 (0.48)	-0.09 (0.69)				
Weight	0.08 (0.73)	-0.03 (0.89)	0.79 (0.00)			
Selwt	0.07 (0.76)	-0.04 (0.88)	0.78 (0.00)	0.99 (0.00)		
7dwt	0.22 (0.36)	-0.16 (0.51)	0.42 (0.06)	0.36 (0.12)	0.35 (0.13)	
Gain	0.07 (0.78)	-0.02 (0.93)	0.78 (0.00)	1.00 (0.00)	0.99 (0.00)	0.29 (0.21)

Rank correlations between SaO and Ln(100-SaO) and between late growth traits were significant ($p<0.05$) and close to unity. Other correlations were not significantly different from zero ($p>0.05$).

Table 4.8. Rank correlations of sire absolute t-values estimated at the position of the putative fleshing score QTL on Ryanodine receptor 3 linkage for all traits. P-values for the hypothesis test of the correlation coefficient being zero are shown in brackets below estimated correlations.

	SaO	Ln(100-SaO)	Flesh	Weight	Selwt	7dwt
Ln(100-SaO)	0.80 (0.00)					
Flesh	0.07 (0.76)	0.21 (0.38)				
Weight	-0.29 (0.22)	-0.05 (0.84)	0.39 (0.09)			
Selwt	-0.35 (0.13)	-0.06 (0.82)	0.34 (0.14)	0.99 (0.00)		
7dwt	0.33 (0.15)	0.28 (0.24)	0.04 (0.89)	0.02 (0.95)	-0.06 (0.81)	
Gain	-0.42 (0.07)	-0.23 (0.33)	0.32 (0.18)	0.96 (0.00)	0.95 (0.00)	-0.08 (0.73)

Analyses allowing for different QTL effects in males and females yielded significant (5% point-wise) F-statistics for SaO and Ln(100-SaO) on RYR1 linkage group, Flesh at all locations tested on RYR3 linkage group and Weight, Selwt and Gain at location 0 cM on RYR3 linkage group. Amongst these, the model including the QTL x sex interaction (QTL x sex) fitted the data better than the model without interaction ($p<0.05$) for SaO, Ln(100-SaO) and Flesh at all locations where the test of QTL x sex vs. no QTL as significant. Table 4.9 shows the F-statistics obtained from the comparison of a model with no QTL and a model with a QTL with sex-different effects and a model with a QTL with sex-equal and a model with a QTL with sex-different effects at all marker locations for SaO and Ln(100-SaO). Table 4.10 shows the same results for fleshing score.

Table 4.9. F statistics obtained from the comparison of a model with no QTL and a model with a QTL with sex-different effects (F_{QTL}) and a model with a QTL with sex-equal and a model with a QTL with sex-different effects (F_{SEX}) at marker locations for blood oxygen saturation (raw –SaO- and transformed -Ln(100-SaO)- data) for all linkage groups (LG). Marker locations are also shown (cM). P_{QTL} and P_{SEX} indicate the significance of the comparisons.

			SaO				Ln(100-SaO)			
LG	Marker	cM	F_{QTL}	P_{QTL}	F_{SEX}	P_{SEX}	F_{QTL}	P_{QTL}	F_{SEX}	P_{SEX}
RYR1	ROS0102	0	2.23	0.01	3.28	0.00	2.41	0.01	3.49	0.00
RYR2	ROS0023	0	0.83	0.76	0.88	0.62	0.81	0.80	0.92	0.56
	ADL0236	1	0.83	0.76	0.88	0.62	0.81	0.80	0.92	0.56
	LEI0237	20	0.85	0.74	0.90	0.59	0.86	0.72	0.99	0.47
RYR3	ROS0013	0	0.88	0.69	0.85	0.66	1.00	0.48	0.93	0.55
	ADL0292	3	0.89	0.66	0.85	0.65	1.02	0.44	0.95	0.52
	ROS0084	6	0.96	0.55	0.89	0.60	1.09	0.32	1.00	0.46
	ADL0312	15	0.95	0.56	0.93	0.55	1.09	0.32	1.01	0.45
	ADL0023, MCW0210	16	0.90	0.65	1.01	0.45	1.03	0.43	1.05	0.40

Table 4.10. F statistics obtained from the comparison of a model with no QTL and a model with a QTL with sex-different effects (F_{QTL}) and a model with a QTL with sex-equal and a model with a QTL with sex-different effects (F_{SEX}) at marker locations for fleshing score for all linkage groups (LG). Marker locations are also shown (cM). P_{QTL} and P_{SEX} indicate the significance of the comparisons.

LG	Marker	cM	F_{QTL}	P_{QTL}	F_{SEX}	P_{SEX}
RYR1	ROS0102	0	0.75	0.70	0.38	0.89
RYR2	ROS0023	0	0.76	0.86	1.04	0.41
	ADL0236	1	0.76	0.86	1.05	0.40
	LEI0237	20	0.80	0.81	1.07	0.38
RYR3	ROS0013	0	2.06	0.00	1.79	0.02
	ADL0292	3	2.08	0.00	1.77	0.02
	ROS0084	6	2.09	0.00	1.78	0.02
	ADL0312	15	2.05	0.00	1.83	0.01
	ADL0023, MCW0210	16	1.83	0.00	1.61	0.04

The highest F-statistic for Flesh on RYR3 linkage group corresponded to marker locus ROS0084 that was the closest marker to the best location identified when the sex interaction was not included. Tables 4.11 and 4.12 show estimates of allele substitution effects for each sire for the putative QTL for SaO, Ln(100-SaO) and Flesh at the best location of a model with sex interaction for analyses without and with QTL x sex interaction. Figures 4.7 and 4.8 show these results graphically. Two sires were inferred to be segregating for a putative QTL with sex-different effects for Ln(100-SaO) ($p<0.05$). Surprisingly, estimates of allele substitution effects were larger for females than for males. Four sires were inferred to be segregating for a putative QTL for fleshing score ($p<0.05$) when no interaction was included in the model, these and three more where inferred to be segregating for a QTL with sex-different effects. For most of the sires inferred to be segregating for the Flesh QTL, estimates of allele substitution effects were larger for males than for females.

Table 4.11. Estimates of allele substitution effects for each sire, in absolute units (α) obtained for blood oxygen saturation (raw –SaO- and transformed -Ln(100-SaO)- data) at the location of ROS0102 on RYR1 linkage group for analyses without and with QTL x sex interaction. The standard error of the estimate (SE) and an indication of the significance of the contrast (P) are also presented. Superscript 1 beside sire name indicate that the sire has been inferred to be segregating (p≤0.05) for a putative Ln(100-SaO) QTL in the analysis including a sex interaction.

	SaO							Ln(100-SaO)						
	No QTL x Sex			QTL x Sex				No QTL x Sex			QTL x Sex			
Sire	α	SE	P	Sex	α	SE	P	α	SE	P	Sex	α	SE	P
594	-1.83	6.06	0.76	Male	-0.88	7.4	0.91	0.04	0.36	0.92	Male	0.01	0.43	0.97
				Female	-1.86	6.17	0.76				Female	0.01	0.36	0.98
616	2.76	5.33	0.60	Male	0.3	6.29	0.96	-0.19	0.31	0.55	Male	0.02	0.37	0.96
				Female	5.27	5.95	0.38				Female	-0.38	0.35	0.28
620 [1]	8.17	5.13	0.11	Male	3.26	5.52	0.56	-0.50	0.30	0.10	Male	-0.19	0.32	0.55
				Female	16.26	6.39	0.01				Female	-1.00	0.37	0.01
622 [1]	8.38	5.22	0.11	Male	6.38	5.95	0.28	-0.56	0.31	0.07	Male	-0.35	0.35	0.32
				Female	9.81	5.41	0.07				Female	-0.70	0.32	0.03
624	-5.65	5.08	0.27	Male	1.22	5.78	0.83	0.23	0.30	0.44	Male	-0.14	0.34	0.67
				Female	-10.02	5.34	0.06				Female	0.46	0.31	0.14
626	-1.95	6.91	0.78	Male	-0.63	7.22	0.93	0.26	0.40	0.51	Male	0.22	0.42	0.60
				Female	-5.66	7.6	0.46				Female	0.48	0.44	0.29

Table 4.12. Estimates of allele substitution effects for each sire (α) for the fleshing score QTL at location 6cM on RYR3 linkage group for analyses without and with QTL x sex interaction. The standard error of the estimate (SE) and an indication of the significance of the contrast (P) are also presented. Superscripts indicate that the sire has been inferred to be segregating for the putative QTL (p≤0.05) in the analysis with no sex interaction (1) or with a sex interaction (2).

	No QTL x Sex Interaction			QTL x Sex Interaction			
Sire	α	SE	P	Sex	α	SE	P
626 [1, 2]	1.22	0.32	<0.001	Male	1.17	0.36	0.00
				Female	1.36	0.41	<0.001
588 [1, 2]	0.76	0.26	0.00	Male	1.07	0.33	0.00
				Female	0.50	0.30	0.10
610 [1, 2]	-0.57	0.25	0.03	Male	-0.67	0.39	0.08
				Female	-0.55	0.29	0.05
620 [1, 2]	0.63	0.28	0.03	Male	0.69	0.33	0.04
				Female	0.59	0.37	0.11
624 [1, 2]	-0.49	0.25	0.05	Male	-0.95	0.35	0.01
				Female	-0.29	0.28	0.30
574	-0.41	0.26	0.11	Male	-0.26	0.31	0.40
				Female	-0.51	0.33	0.12
592 [2]	0.35	0.27	0.19	Male	0.08	0.33	0.81
				Female	0.62	0.32	0.05
594	-0.41	0.29	0.15	Male	-0.54	0.44	0.22
				Female	-0.39	0.31	0.20
569 [2]	0.21	0.25	0.40	Male	0.77	0.40	0.05
				Female	-0.02	0.28	0.93
618	-0.22	0.26	0.39	Male	-0.07	0.30	0.82
				Female	-0.47	0.36	0.19
616	0.26	0.29	0.39	Male	0.65	0.38	0.09
				Female	-0.08	0.35	0.82
581	0.17	0.28	0.55	Male	0.10	0.33	0.75
				Female	0.25	0.32	0.43
627	-0.21	0.28	0.47	Male	0.22	0.47	0.64
				Female	-0.39	0.31	0.21
622	-0.14	0.29	0.63	Male	-0.06	0.38	0.87
				Female	-0.24	0.31	0.45
602	-0.16	0.34	0.64	Male	0.40	0.41	0.34
				Female	-0.57	0.38	0.13
612	0.16	0.25	0.52	Male	0.15	0.36	0.68
				Female	0.15	0.27	0.57
580	-0.05	0.25	0.86	Male	0.09	0.29	0.76
				Female	-0.24	0.33	0.48
605	-0.04	0.27	0.90	Male	0.07	0.34	0.83
				Female	-0.11	0.30	0.72
619	0.02	0.29	0.94	Male	0.64	0.36	0.07
				Female	-0.50	0.34	0.14
579	-0.01	0.25	0.96	Male	0.46	0.33	0.17
				Female	-0.38	0.30	0.21

Figure 4.8. Estimates of allele substitution effects for each sire obtained for blood oxygen saturation at the location of ROS0102 on RYR1 linkage group for analyses without (squares) and with QTL x sex interaction (triangles and dots for allele substitution effect in males and females respectively). Full symbols are for raw data (SaO) and empty symbols are for transformed (Ln(100-SaO)) data. Red crosses on the x axes represent sires inferred to be segregating ($p \leq 0.05$) for a putative SaO (x) or Ln(100-SaO) (+) QTL in the analysis including a sex interaction.

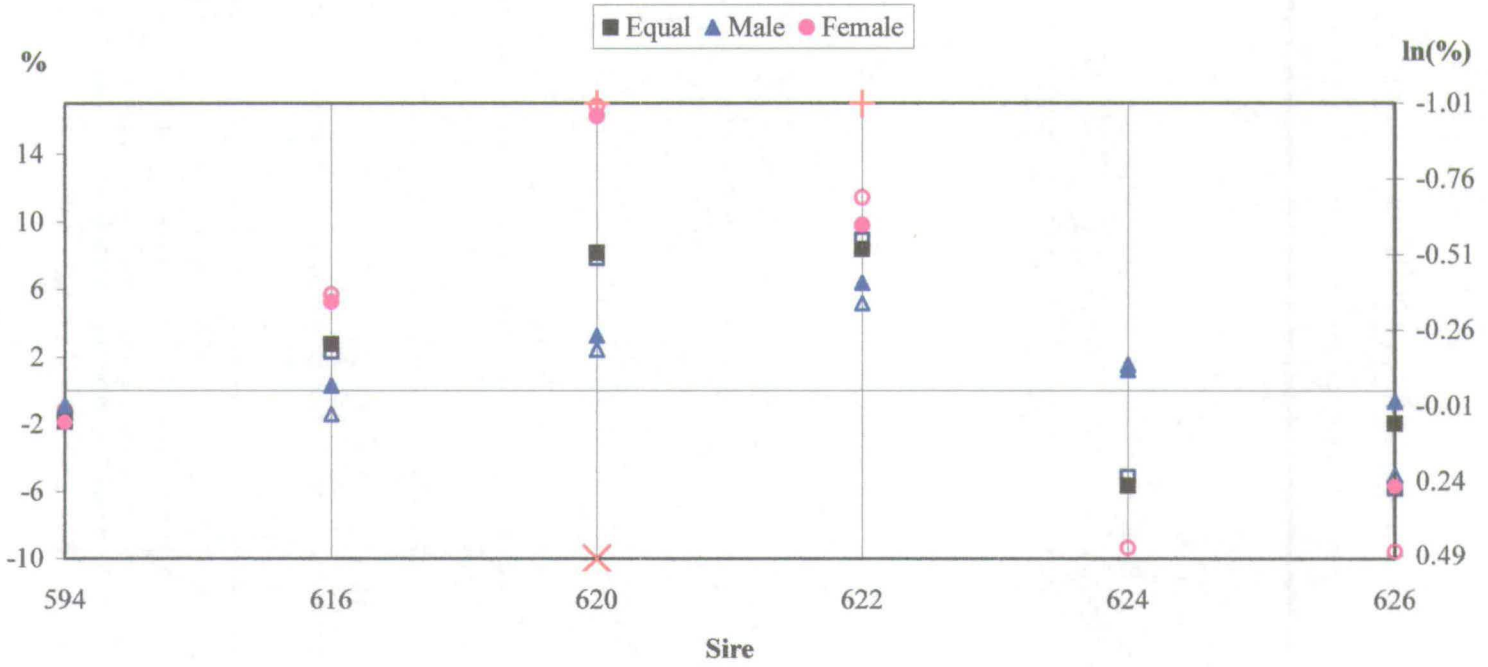
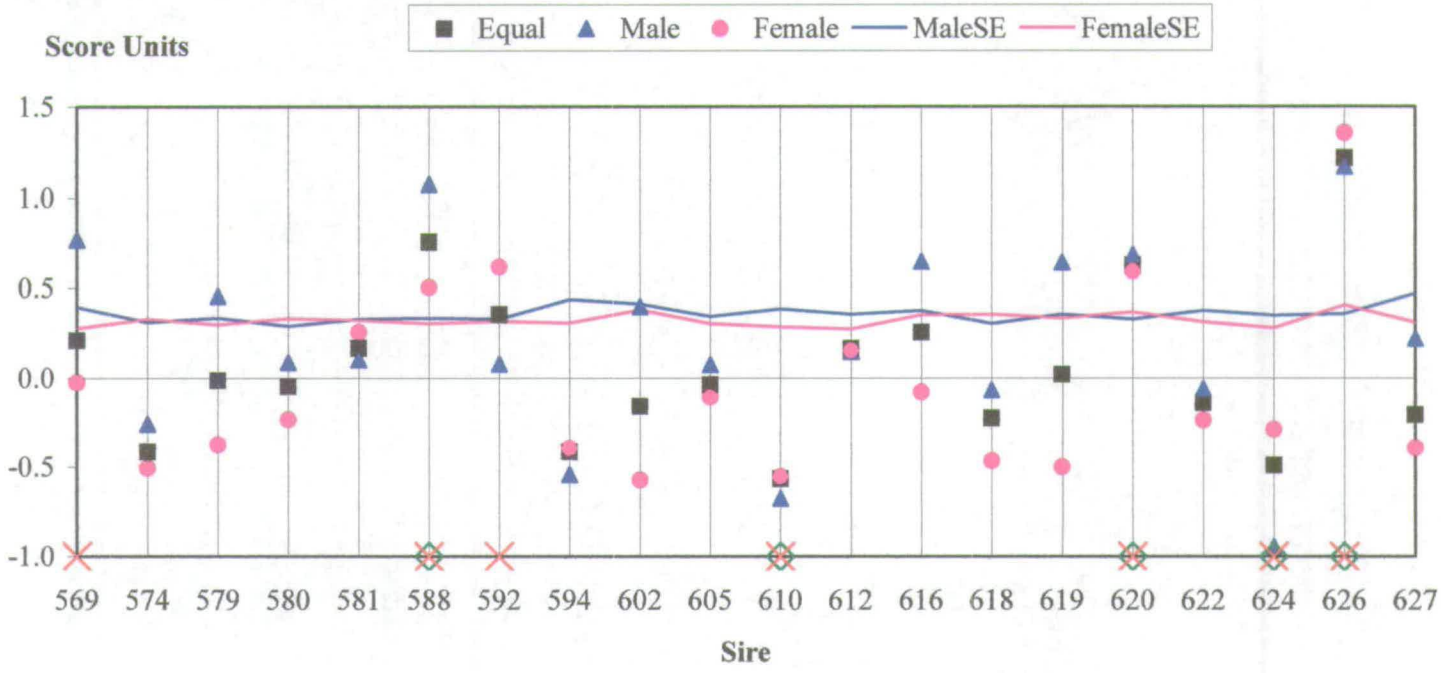


Figure 4.9. Estimates of allele substitution effects for each sire (α) for the fleshing score QTL at location 6cM on RYR3 linkage group for analyses without (squares) and with QTL x sex interaction (triangles and dots for allele substitution effect in males and females respectively). The standard errors are also presented for estimates of male and female allele substitution effects. Red crosses on the x axes represent sires inferred to be segregating (p≤0.05) for a putative QTL in the analysis including a sex interaction and green circles represent sires inferred to be segregating in the analysis with no sex interaction.



4.4 Discussion

The current study was set up to study the genetic basis of SaO (an indicator of ascites susceptibility). We followed a candidate gene approach and looked at markers associated with Ryanodine receptors, since previous evidence suggested these genes were strong candidates. Twenty sire-families were selected from the breeding population to create a mapping population. These 20 sire-families were selected from 50 on basis of high probability of being heterozygous for a putative major gene affecting SaO identified in previous studies and a total of 59 offspring per family (equal numbers of high and low within family SaO MST values) were selected for genotyping, together with the 20 sires. We have found indications that a QTL linked to RYR1 with a large effect on SaO (and Ln(100-SaO)) in females may be segregating in this population. Nonetheless, the results obtained for this region are based on small numbers of birds (six sires with on average 25 informative offspring each) and should be taken with caution. Mutations in RYR1 have been linked to malignant hyperthermia in humans (see for example Quane *et al.* (1993)) and pigs (Fujii *et al.*, 1991) and with skeletal muscle dysgenesis in chickens (Airey *et al.*, 1993). Further research is needed to elucidate if RYR1 (or linked genomic regions) are associated with blood oxygen saturation levels. A possible way forward would be the use of more informative markers in the region. No evidence of linkage was found for SaO or Ln(100-SaO) on the RYR2 and RYR3 regions. Information content within these regions was reasonably high as was the number of sire-families used in the analyses. In our study, we assumed that RYR2 mapped to a region of the q arm of GGA2 as suggested in Schmid *et al.* (2000). Comparative mapping information would suggest that RYR2 could in fact be located on the q arm of chromosome 3. RYR2 maps to the q41-q43 human chromosome 1, that also contains the ACTN2 gene (closer to the centromere than RYR2), located only about 0.25 Mb from RYR2 in humans (see http://genome.ucsc.edu/cgi-bin/hgTracks). There is evidence of conserved synteny for RYR2 and ACTN2 in mice and pigs (see, for example, http://www.informatics.jax.org/searches/linkmap.cgi?chromosome=13&midpoint=7.00&cmr

ange=1.0&dsegments=1&syntenics=0 for mice and http://www.thearkdb.org/browser for pigs). Based on this information, it is likely that synteny is also conserved in chicken. Suchyta *et al.* (2001) genetically mapped ACTN2 in chickens to chromosome 3 and showed the existence of conserved synteny between human and chicken for genes on HSA1q42-43 (ADPRT, TGF-beta2 and ACTN2). Unfortunately, no genes closely linked to RYR2 in the most telomeric region of band 1q43 in humans have yet been mapped in chicken, and there is no proof of conserved synteny between humans and chickens for this region. Given that here is strong suggestion on comparative basis that RYR2 could map to a different location that the one reported on the literature, we considered it necessary to verify that RYR2 mapped to GGA2. The first verification step involved FISH-mapping the BAC that supposedly contained RYR2. All work related to this was carried out under the supervision of Jacqueline Smith at Roslin Institute. This confirmed that the BAC contained sequence from GGA2. The second verification step, carried out by others also at Roslin Institute, involved sequencing the BAC to confirm that it contained DNA sequence from RYR2 and from MCW0096. Several attempts to do this have been made using different approaches, but unfortunately technical difficulties arose and no results could be produced (R. Paton, personal communication). We could therefore not verify nor refute that RYR2 is contained in GGA2, and are not certain that our linkage analysis really refers to the RYR2 region. Further work is necessary to clarify this point and at the moment we do not have evidence to state that RYR2 has no effect on blood oxygen saturation levels.

We detected a QTL with a mean standardised effect size in segregating sires of 0.89 (0.37 if averaging over all sires) for fleshing score on RYR3 linkage group. This effect size is similar to the one assumed in our power study. Assuming equal QTL effect sizes for males and females, four out of 20 sires were inferred to be segregating at the QTL. That translates to a frequency of 0.20 heterozygous, that is less than half that assumed in the power study (0.45). Allowing sire allele substitution effects to differ between males and females significantly improved the fit of the model and allele substitution effects were generally

inferred to be larger for males than for females. Weaker evidence of QTL for late growth (Weight, Selwt and Gain) was also found on RYR3, but sire families segregating for this growth QTL do not seem to be the same that were inferred to be segregating for the Flesh QTL, supporting the hypothesis that two linked QTL may exist on GG5, one affecting late growth and another one affecting breast conformation. Growth and conformation traits have long been selection criteria in broiler breeder populations. One could therefore expect that QTL with large effects affecting these traits would be fixed in these populations. De Koning *et al.* (2003) detected QTL for growth in a pedigree of commercial broilers on GGA4 and suggested that QTL for traits that have been long selected for in these populations could still be segregating because they may have pleiotropic effects on fitness traits.

Breast meat is the most valuable carcass part in chicken and a QTL improving relative breast-related production traits would be of interest to the breeding industry. McElroy *et al.* (2002) reported associations of marker MCW0193 on GG5 with growth and breast-related traits – but not fleshing score - in a cross between two broiler lines. MCW0193 is located about 40 cM away from ROS0013 on the consensus linkage map, and was the only marker on GGA5 genotyped in their study. MCW0193 is closely linked to the genes TH, IGF2 and INS, and this region has been shown to control growth related traits in several species (see, for example, Gu *et al.* (2002) for humans and Nezer *et al.* (2002) for pigs). This region also contains MYOD1 and recent work in mice (Spiller *et al.*, 2002; Langley *et al.*, 2002) has shown that this locus interacts with the myostatin locus to control the processes of muscle cell proliferation and differentiation. Mutations in the myostatin locus have been linked to double-muscling in several cattle breeds (see, for example, Dunner *et al.* (2003)) and increased total muscle mass in mice (McPherron *et al.*, 1997). Guernec *et al.* (2003) suggested that the myostatin locus was implicated in differences in breast meat yield between a line of chickens selected for high breast meat yield and a control line and we hypothesise that MYOD1 could participate in this through interactions with the myostatin locus. McMahon *et al.* (2003) also showed that decreased expression of myostatin in male

mice compared to females was associated with sexual dimorphism (males have comparatively greater muscle mass than females). We point out MYOD1 as an interesting candidate for further studies of the genetic basis of growth and breast conformation traits in chickens. TGF-beta3, a member of the transforming growth factor beta superfamily, is closely linked to the RYR3 region we studied on GG5. Type beta transforming growth factors are polypeptides that act hormonally to control the proliferation and differentiation of multiple cell types. Li *et al.* (2002) and Li *et al.* (2003) studied a series of production traits, anatomical and physiological measures in two resource populations and identified associations of TGF-beta3 with body weight, breast muscle weight, percentage abdominal fat, spleen and liver weight, several bone characteristics and blood content of T3 and IGF2 in a resource population derived from a cross of a broiler and a Leghorn line. They suggested that the broiler allele was dominant for growth traits and breast muscle mass. In a different population (broiler x fayoumi cross) they found associations of TGF-beta3 with similar characters. Sewalem *et al.* (2002) also reported a suggestive genome-wide association of a region of GG5 with growth at three weeks. Other examples of traits linked to TGF-beta3 are disorders in lung formation in mice (Kaartinen *et al.*, 1995) and male infertility in rats (Liu *et al.*, 2003), both of interest in meat type chickens. CAPN1, also linked to the RYR3 region studied, has been associated with meat tenderness in cattle (Page *et al.*, 2002). In chapter five, we have found evidence of genome-wide suggestive linkage for Troponin T concentration with the region of GGA5 harbouring RYR3 and suggested that this region deserves further attention in metabolic disorder studies. The current study further supports that GGA5 merits further study since it may harbour QTL that affect both production and health characters. If these QTL were segregating in commercial breeding populations, an understanding of the relationships between these QTL would needed in order to use these QTL in breeding programmes.

4.5 Conclusion

Over the past decade, the importance of inherited gene defects in the pathogenesis of primary cardiomyopathies has been recognized, with mutations in some 18 genes having been identified as causing hypertrophic cardiomyopathy and/or dilated cardiomyopathy in humans (Fatkin and Graham, 2002), amongst them RYR2. Given physiological differences between birds and mammals, we set out to study the putative association of the whole RYR gene family with ascites in a broiler population, through the use of SaO, a predictor and indicator of the disorder. No strong evidence of association was found in this study, but further work on RYR1 and RYR2 is necessary to rule out their implication in the genetic control of SaO. Nonetheless, we are aware that the RYR gene family is only a small part of a wealth of candidate genes/gene families that may be involved in the genetic control of ascites. Other strong candidates are other genes found to be involved in pulmonary hypertension in other species (Wideman and French (2000)), for example BMPR-2, a member of the transforming growth factor family of receptors, has been pointed out as it underlies many familial and apparently sporadic cases of primary pulmonary hypertension (*e.g.* Morrell and Wilkins (2001)). Other authors (*e.g.* Scheele *et al.* (1996), Decuypere *et al.* (2000), Luger *et al.* (2001)) have noted malfunctions of the thyroidal axis in ascitic birds, and therefore genes involved in the control of this axis are also candidates that merit study. Ultimately, a genome scan could be carried out using the resource population created for this study that would provide a more complete vision of genomic regions that play an important role in the genetic control of blood oxygen saturation levels and the ascites syndrome.

CHAPTER FIVE

5 QUANTITATIVE TRAIT LOCI AFFECTING ORGAN WEIGHTS AND BLOOD PARAMETERS IN CHICKEN

5.1 Introduction

In chapter four, we have used a collection of half-sib families from a broiler population to test for linkage of SaO and production traits with markers in candidate regions. Such a design allows for the identification of markers or genomic regions (QTL) responsible for within-population variation. Marker-trait associations identified in this way could potentially be directly used in marker-assisted selection (MAS) in ongoing breeding programmes to achieve more rapid improvement. An alternative approach to identify marker trait-associations is the use of experimental crosses of inbred or outbred lines that differ for a number of traits of interest. These designs allow for the identification of genomic regions responsible for between-population variation. Traditional breeds usually have a poorer performance in production traits than breeds used in intensive production, but generally are superior for specific traits. For example, Meishan pigs are more prolific than Large White pigs (see Haley *et al.* (1995)), but grow slower than commercial western pigs and have, for western standards, an undesirable carcass composition. In such cases, the identification of genomic regions that confer superior qualities to traditional breeds would allow their introgression in commercial breeds (marker assisted introgression (MAI)). On occasions, QTL identified in population crosses would also be segregating within commercial populations and could also be used in MAS. In general, the identification of marker-trait associations represents a first step towards the identification and cloning of the genes controlling the characters studied.

In this study, we used an F_2 population derived from the cross of a broiler and a layer line from which phenotypes were available for a series of traits. Although broilers and layers have both been heavily selected for decades, selection has been done on different traits. Broilers have been, traditionally, heavily selected on growth and conformation traits while

layers have mainly been selected on egg-production traits. As a result, broilers and layers differ substantially in size, muscling and reproductive fitness, but they also differ in traits for which selection has not been consciously made, such as susceptibility to heart and lung disorders (like ascites and sudden death) and skeletal muscle abnormalities, more commonly suffered by broilers. The main purpose of this study was to investigate if QTL that affect the birds' susceptibility to these pathologies were segregating in this F_2 population. To this aim, we analysed a series of traits that are know to vary in birds that suffer from heart, lung or muscular dysfunction as well as some traits that are not involved in cardiopulmonary or muscular disorders, that were used as control traits.

Hocking *et al.* (1985) and Deeb and Lamont (2002) observed that differences in live weight between broilers and layers were generally reflected in all measures of weight and size, but broilers have relatively longer intestines and the relative weight of other organs is higher in layers. Emmans and Kiryazakis (2000) postulated that, in fast growing birds, the development of the heart and other supply organs is penalised due to the energetic needs of the growing muscle and that this is at the origin of metabolic disorders and tissue and organ dysfunction in fast growing chickens. Mitchell and Sandercock (1995) showed that the concentration of creatinine kinase in avian blood can be used as an indicator of skeletal muscle dysfunction or damage and Sandercock *et al.* (2001) pointed out that there are differences in creatinine kinase activity between broilers and layers that cannot be explained only by differences in live weight. Maxwell *et al.* (1994) observed that ascitic broilers exhibit higher levels of troponin T (which is an indicator of early myocardial damage) than healthy birds. Ascitic birds also show raised packed cell volume (PCV) and red and white blood cell count, as well as increased (although not different at a 5% significance level) mean cell volume (MCV) (Maxwell *et al.*, 1986). Furthermore, Maxwell *et al.* (1990b) found that broilers have higher PCV and more red blood cells than layers, but they did not observe significant differences in MCV between strains.

We carried out a series of genome scans looking for a single or two QTL per linkage group using the least squares method developed by Haley *et al.* (1994) to analyse data from F_2 populations derived from crosses between outbred lines. We searched the autosomes for Mendelian and imprinted QTL, and for QTL with sex-different effects. The Z chromosome was also scanned. QTL identified on linkage groups not being searched were included in the analyses as cofactors following Knott *et al.* (1998) and confidence intervals for QTL locations were obtained by bootstrapping (Visscher *et al.*, 1996).

5.2 Materials and methods

5.2.1 Mapping population and traits

Levels of creatinine kinase and troponin T, total blood cell count (TBCC), PCV, weights of the dressed carcass and several organs (liver, heart, spleen and gizzard) and intestine length were recorded in an F_2 population derived from the cross of two males and two females from both a large broiler line and a small egg-laying line. The F_1 consisted on eight males and 31 females that were crossed to produce an F_2 of over 500 birds (for more details on the mapping population see Sewalem *et al.* (2002)).

All traits were recorded at nine weeks except creatinine kinase, recorded at six weeks. Phenotypes of between 461 and 314 F_2 birds were available depending on the trait (see Table 5.1). Pedigree, phenotypic and marker information was stored in http://www.resspecies.org (Law and Archibald, 2000).

Table 5.1. Acronyms, number of records, means and residual standard deviations (F_2sd) for the traits analysed.

Trait	Acronym	#F_2 records	Mean	F_2sd
Creatinine kinase concentration (IU/l)	-	451	208.78	131.96
(Ln-transformed trait analysed)	LNCREAT	451	5.18	0.39
Troponin T concentration (ng/ml)	-	445	0.04	0.20
(Ln-transformed trait analysed)	LNTROP	445	-3.47	0.41
Packed Cell Volume (%)	PCV	313	28.80	1.70
Total blood cell count (10^6/mm^3)	TBCC	314	2.35	0.16
Mean cell volume (μm^3)	MCV	312	123.27	7.15
Heart weight (g)	HEART	461	10.79	1.59
Dressed Carcass weight (g)	CARCASS	461	1350.10	159.78
Liver weight (g)	LIVER	461	39.95	3.92
Spleen weight (g)	SPLEEN	461	4.25	0.80
Gizzard weight (g)	GIZZARD	461	29.00	4.51
Intestine length (cm)	INTESTINE	461	162.60	11.46

5.2.2 Genotyping and Linkage map

For details on genotyping see Sewalem *et al.* (2002).

The linkage map was constructed using Cri-map (Green *et al.*, 1990) in a previous study (X. Yu, unpublished). It consisted of 101 markers scattered across 26 linkage groups. The total map length was 2503 cM and map distances were assumed to be equal for males and females in the analyses. Table 5.2 shows details on the linkage map used. The mean distance between consecutive markers on a linkage group was 42.5 cM, ranging from 0.2 to 100 cM.

Table 5.2. Summary of map data for all linkage groups analysed.

	Linkage group	# of markers	Map length (cM)
	1	24	555
	2	12	398
	3	11	259
	4	4	202
	5	6	180
	6	4	88
	7	3	109
	8	2	94
	9	4	153
	10	1	0
	11	5	71
	12	2	33
	13	3	76
	14	1	0
	15	2	45
	17	1	0
	18	2	23
	23	3	51
	24	1	0
	26	1	0
	27	1	0
	28	2	39
	E25C31	1	0
	E32	1	0
	E38	1	0
	Z	3	127
Totals	**26**	101	2503

5.2.3 Statistical analyses

5.2.3.1 *Basic least squares model*

The analyses for creatinine kinase and troponin T concentration were carried out on the natural logarithm of the original observations since the trait distributions on the transformed scale were closer to a normal distribution than untransformed data. For all traits analysed, the fixed effects of sex, F_2 family and pen were fitted. Except for creatinine kinase, for which we fitted live weight at six weeks as a covariate, all the other traits were analysed including dressed carcass weight in the basic model. Troponin T was adjusted for assay tube and assay number effects. Observations for which the standardised residuals exceeded four after correction for these fixed effects were removed from the dataset. The maximum number of birds removed from the dataset was six, and that was for the transformed troponin T concentration data. The statistics presented in Table 5.1 were obtained after removing these observations.

5.2.3.2 *QTL analyses and confidence intervals for QTL locations*

QTL analyses were conducted using a least squares framework, following the method developed by Haley *et al.* (1994) for F_2 populations.

This method assumes that the grandparental lines used to derive the F_2 are fixed for alternative QTL alleles (Q and q), but may be segregating at marker locations.

The analyses are carried out in two steps. First, the probabilities of each F_2 individual of being each of the four possible QTL genotypes (QQ, Qq, qQ and qq, where the first allele is inherited from the male parent and the second allele from the female parent) are computed for each location in the genome using multiple marker genotypes (see Haley *et al.* (1994)). Secondly, for each location, trait values are regressed on linear combinations of these probabilities, which allows to estimate the additive (a), dominance (d) and imprinting (i) effects for a putative QTL at each location. a is the effect of QQ and qq has an effect of $-a$. For details on the parameterisation see Knott *et al.* (1998). Genotype probabilities can also

be used to check for marker information content and segregation distortion (Knott *et al.*, 1998).

Markers on chromosome Z appeared to belong to a region that did not recombine with the W chromosome (*i.e.*, they were outside any pseudoautosomal region) and this was taken into account for the computation of QTL genotype probabilities for this sex chromosome. Males carry two copies of any putative QTL on chromosome Z and they can have all possible QTL genotypes (*QQ*, *Qq*, *qQ* and *qq*), so that a model with additive dominance effects can be fitted. Females carry only one copy of the putative QTL and only the effect of being *QW* vs. *qW* (where *W* could originate from either broilers or layers) can be estimated.

In a first stage, each linkage group was searched for a single QTL with *a* and *d* effects and a single QTL with *a*, *d* and *i* effects, firstly assuming sex-equal and then assuming sex-different effects (*i.e.*, with the QTL having the same effect in males and females or not). The model including a QTL was compared with a model without a QTL using an F ratio. For each linkage group, the location showing the highest F ratio was considered the most likely location for a QTL on this linkage group. If the test statistic at this "best location" exceeded the (model-dependent) genome-wide threshold for suggestive linkage (see below) for only one of the QTL models fitted, this model was chosen for further analyses (*i.e.*, searches including background genetic effects). If the test statistic for more that one model exceeded the suggestive linkage threshold, and the best location for the QTL was (roughly) the same, since the models are nested, we could test which of them fitted best the data. If the best location across significant models could not be considered to be the same we tested the models against each other at both best locations and, generally, we used the model with fewer parameters in further analyses. Searches for two QTL simultaneously per linkage group were also carried out.

In a second stage, the searches were repeated for all linkage groups that showed suggestive or significant linkage (see below), including in the basic model, for each given

trait, the QTL identified in other linkage groups. The inclusion of unlinked QTL would take account of unlinked genetic variation and reduce the residual variance, potentially increasing power and removing biases in QTL parameter estimates (Jansen, 1993; Zeng, 1993).

Finally, confidence intervals for QTL locations were obtained by bootstrapping (Visscher *et al.*, 1996). A thousand resamples were used and the 95% confidence intervals were the regions for which the 950 less extreme samples were obtained.

We carried out additional searches using a model for which we allowed the QTL to have different effects across F_2 families. This could be observed if one or both grandparental lines were segregating at the QTL.

Chromosome 1, a very long chromosome, was analysed in two overlapping segments covering 342 cM (from position 0 cM to position 342 cM) and 346 cM (from position 199 cM to position 555 cM) respectively.

The module F_2 QTL analysis from QTL Express (Seaton *et al.*, 2002) implements the method developed by Haley *et al.* (1994) and was used to perform genome scans looking for a single or two QTL per linkage group and tests for linkage at single positions, as well as to obtain the confidence intervals for QTL locations. FORTRAN programs have been used for specific analyses.

5.2.3.3 Significance thresholds

Genome-wide significance thresholds (assuming a QTL with *a* and *d* effects, *i.e.* for a model with 2 degrees of freedom (df) for the numerator, ∞ for the denominator) were obtained by permutation using a simulated data set in a previous study (see Sewalem *et al.* (2002) for details). The genome-wide threshold for suggestive linkage (where we expect to obtain, by chance, one significant result per genome scan) is 5.0 and the 5% and 1% genome-wide significance thresholds are 8.2 and 10.0, respectively (see Lander and Kruglyak (1995)).

Approximate significance thresholds for alternative single QTL models (with sex interaction and/or imprinting) can be obtained from a standard F distribution table. The F

ratio threshold obtained by simulation for the model with an additive and dominance component (2 df for the numerator and ∞ df for the denominator) corresponds to a tabulated probability (α) under a standard F distribution (2 df/∞ df). The tabulated critical value for a standard F distribution with *x* df/∞ df for α can be used as approximate significance threshold for a QTL model where *x* QTL components are estimated. For example *x*=3, for a model where the QTL has an additive, a dominance and an imprinting component, *x*=4 if we fit a QTL with additive and dominance effects and sex interaction. Nested single QTL models were compared using the nominal point-wise significance.

No thresholds were obtained empirically to test for the presence of two vs. no QTL. Instead, we used genome-wide suggestive thresholds obtained for single QTL searches adjusted for df as described above. To test for the presence of two vs. one QTL we used again this threshold as suggested by Spelman *et al.* (1996) and empirically validated for a particular data set by De Koning (2001).

5.3 Results

5.3.1 Descriptive statistics of traits

Table 5.1 shows the total number of F_2 birds with phenotypic records, means and residual standard deviations of all traits, after removal of outliers.

5.3.2 Significance thresholds

The significance thresholds used are presented in Table 5.3.

Table 5.3. Point-wise and genome-wide significance thresholds.

	Point-wise	Genome-wide		
	5% significance	Suggestive linkage P < 0.007	5% significance P < 0.0003	1% significance P < 0.0001
1 df / ∞ df	3.9	7.3	13.3	15.4
2 df / ∞ df	3.0	5.0	8.2	10.0
3 df / ∞ df	2.6	4.1	6.4	7.2
4 df / ∞ df	2.4	3.6	5.4	6.3
6 df / ∞ df	2.1	3.0	4.3	4.8
8 df / ∞ df	2.0	2.7	3.7	4.1
58 df / ∞ df	1.4	1.6	1.9	2.0

5.3.3 Single QTL analyses

Table 5.4 shows the location with the highest test statistic and the estimates of the QTL effects at this location for linkage groups with genome-wide suggestive or significant results for models with sex-equal effects. If a model with sex-different effects fitted the data best, no results are presented at this table. Test statistics, 95 % confidence intervals, marker brackets for QTL locations and the percentage of the variance accounted for by the QTL are also presented. Table 5.5 shows the same results for models with sex-different effects. A positive additive estimate means that the QTL alleles coming from the broiler line increase the trait value and a positive imprinting estimate means that inheriting the broiler QTL allele through the male parent increases the trait value.

Table 5.4. Single QTL results for QTL with sex-equal effects. The location with the highest test statistic and the estimates of the QTL effects (and standard errors) at this location for linkage groups (LG) with genome-wide suggestive or significant results are shown, together with test statistics (F ratio), 95 % confidence intervals (95% CI) and marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE).

Trait	LG	F ratio	(1)	Location (cM)	95% CI	QTL Effects (se)			%VE (2)	Flanking markers	
						a	*d*	*i*			
LNCREAT	4	4.6	+	63	0-201	-0.13 (0.05)	-0.07 (0.11)	-0.1 (0.05)	2.7	ROS0015	ADL0266
LNCREAT	9	4.6	+	0	0-152	0.04 (0.03)	0.10 (0.04)	-0.08 (0.04)	2.7	ROS0078	MCW0135
LNCREAT	11	5.5	+	0	0-70	-0.08 (0.03)	0.08 (0.04)	-	2.2	MCW0097	LEI0110
LNTROP	2	7.2	+	244	84-341	-0.06 (0.04)	0.27 (0.08)	-	3.6	ADL0196	LEI0127
LNTROP	5	5.8	+	127	0-153	0.23 (0.07)	-0.36 (0.26)	-	2.8	ROS0084	ADL0298
LNTROP	11	8.7	*	22	3-61	-0.07 (0.03)	0.16 (0.04)	-	4.4	ROS0111	ADL0308
PCV	14	4.3	+	0	-	-0.18 (0.20)	0.35 (0.38)	1.06 (0.30)	3.7	-	-
TBCC	1	4.7	+	1	0-348	0.03 (0.02)	0.06 (0.02)	0.04 (0.02)	4.3	MCW0168	ADL0160
TBCC	2	10.1	**	114	67-273	0.08 (0.02)	0.07 (0.03)	-	6.6	ADL0176	ROS0018
TBCC	6	7.1	+	88	17-88	0.05 (0.01)	0.04 (0.02)	-	4.5	ADL0142	ADL0323
MCV	2	6.8	+	115	100-298	-2.67 (0.86)	-2.99 (1.38)	-	4.0	ADL0176	ROS0018
MCV	14	4.8	+	0	-	0.64 (0.84)	1.16 (1.58)	4.26 (1.26)	4.2	-	-
HEART	1	5.0	+	101	0-322	0.51 (0.22)	1.30 (0.65)	-	2.0	MCW0010	ADL0180
HEART	9	6.2	+	152	27-152	-0.38 (0.11)	0.08 (0.16)	-	2.6	ROS0030	MCW0134
CARCASS	1	7.4	+	191	35-202	45.29 (11.91)	22.89 (21.11)	-	3.1	LEI0146	MCW0018
CARCASS	1	13.8	**	429	403-479	53.47 (11.27)	46.07 (18.02)	-	6.0	LEI0106	ADL0183
CARCASS	2	7.3	+	268	65-321	36.87 (9.63)	-1.17 (13.66)	-	3.1	LEI0127	LEI0147
CARCASS	3	8.7	*	181	72-204	61.67 (14.81)	1.30 (35.17)	-	3.7	MCW0187	ADL0306
CARCASS	4	29.9	**	147	135-161	171.18 (22.15)	2.55 (70.69)	-	12.7	ADL0266	LEI00733
CARCASS	8	9.1	*	46	0-94	113.32 (26.57)	-72.42 (98.63)	-	3.9	ADL0179	ROS0075
CARCASS	13	10.9	**	60	36-76	58.62 (14.13)	41.95 (27.98)	-	4.7	ADL0147	ADL0225
CARCASS	27	18.3	**	0	-	67.64 (11.23)	-17.87 (15.49)	-	8.0	-	-
LIVER	1	6.3	+	417	360-541	1.06 (0.31)	0.45 (0.45)	-	2.6	LEI0106	MCW0036

Trait	LG	F ratio	(1)	Location (cM)	95% CI	QTL Effects (se)			%VE (2)	Flanking markers	
						a	*d*	*i*			
LIVER	4	5.4	+	102	0-139	0.66 (0.32)	0.81 (0.57)	1.12 (0.36)	3.2	ADL0266	LEI0073
LIVER	15	5.5	+	39	10-45	-1.31 (0.44)	-1.19 (0.93)	-	2.2	LEI0083	MCW0080
LIVER	24	7.1	+	0	-	-1.37 (0.39)	-1.17 (1.00)	-	3.0	-	-
SPLEEN	1	8.7	*	189	147-238	-0.29 (0.07)	-0.08 (0.12)	-	3.6	LEI0146	MCW0018
GIZZARD	1	5.4	+	201	8-338	-1.11 (0.37)	-0.98 (0.60)	-	2.2	LEI0146	MCW0018
INTESTINE	11	4.6	+	40	3-70	-2.43 (0.98)	1.19 (1.79)	3.02 (1.11)	2.7	ROS0111	ADL0308
INTESTINE	14	7.8	+	0	-	3.82 (1.06)	-3.56 (1.90)	-	3.4	-	-

(1) + indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level respectively.

(2) Variance explained by individual QTL obtained as the percent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

Table 5.5. Single QTL results for QTL with sex-different effect. The location with the highest test statistic and the estimates of the QTL effects (and standard errors) at this location for linkage groups (LG) with genome-wide suggestive or significant results are shown, together with test statistics (F ratio), 95 % confidence intervals (95% CI) and marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE).

Trait	LG	F ratio	(1)	Location (cM)	95% CI	(2)	QTL Effects (se)			%VE (3)	Flanking markers	
							a	*d*	*i*			
LNCREAT	1	3.6	+	255	208-555	M	-0.11 (0.05)	0.18 (0.09)	-0.01 (0.05)	3.8	ADL0319	LEI0101
						F	0.08 (0.05)	0.07 (0.09)	0.16 (0.05)			
LNTROP	17	4.4	+	0	-	M	-0.16 (0.06)	-0.33 (0.12)	-	4.0	-	-
						F	0.01 (0.06)	0.18 (0.11)	-			
PCV	1	4.8	+	340	163-516	M	0.17 (0.22)	-0.87 (0.32)	-	5.4	LEI0088	ROS0081
						F	0.67 (0.22)	0.34 (0.34)	-			
PCV	2	3.7	+	114	0-397	M	0.77 (0.28)	0.47 (0.45)	-0.85 (0.25)	5.8	ADL0176	ROS0018
						F	0.14 (0.28)	-0.04 (0.46)	0.49 (0.25)			
TBCC	11	3.8	+	52	0-70	M	-0.03 (0.02)	0.05 (0.03)	-	4.3	ROS00111	ROS0112
						F	0.02 (0.02)	-0.10 (0.03)	-			
HEART	1	4.6	+	489	213-555	M	-0.98 (0.23)	-0.03 (0.43)	-	3.6	LEI0079	ROS0025
						F	-0.05(0.21)	0.83 (0.43)	-			
HEART	13	5.5	*	67	0-76	M	-0.51 (0.20)	-1.25 (0.38)	-	4.5	ADL0147	ADL0225
						F	-0.22(0.21)	0.03 (0.37)	-			
LIVER	Z	6.2	+	36	0-106	M	0.18 (0.82)	2.18 (1.03)	-	3.8	ROS0072	LEI0111
						F	2.12 (0.75)	-	-			
GIZZARD	2	7.7	**	114	43-243	M	-2.94 (0.59)	0.63 (0.97)	-	6.3	ADL0176	R0S0018
						F	-0.24 (0.59)	1.90 (0.95)	-			
GIZZARD	5	3.8	+	95	0-149	M	-2.40 (0.63)	-0.50 (0.97)	-	2.7	ROS0084	ADL0298
						F	-0.21 (0.62)	0.70 (1.28)	-			
INTESTINE	Z	6.2	+	108	3-127	M	5.15 (1.71)	-4.64 (2.06)	-	3.8	LEI0111	LEI0075
						F	1.47 (1.62)	-	-			

(1) + indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level respectively.
(2) Sex: M is male and F female.
(3) Variance explained by individual QTL obtained as the percent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

5.3.3.1 Results for QTL with sex-equal effects

We identified two QTL significant at a genome-wide level for health-related traits (LNTROP on chromosome 11 and TBCC on chromosome 2). In addition, six genome regions located on chromosomes 1, 3, 4, 8, 13 and 27 showed evidence of significant linkage at a genome-wide level for dressed carcass weight and one in chromosome 1 was significant for spleen weight. Several regions showed suggestive evidence of linkage for LNCREAT, LNTROP, PCV, TBCC, MCV, heart, carcass, liver and gizzard weight and for intestine length.

These QTL acted mainly in an additive fashion. For TBCC and carcass weight the broiler allele always increased the trait value whereas for the rest of the traits there was not such a clear pattern. The dominance component was only statistically different from zero (5% significance level) for the QTL identified for LNCREAT on chromosomes 9 and 11, LNTROP on chromosomes 2 and 11, TBCC (chromosomes 1, 2 and 6), MCV on chromosome 2 and one of the two QTL identified on chromosome 1 for carcass weight. For all except LNTROP, the broiler allele was dominant.

The first scans revealed a series of suggestive or significant locations for which a model that included an imprinting effect was the only model for which the test statistic exceeded the genome-wide suggestive threshold (LNCREAT on chromosomes 1 and 9, PCV on chromosomes 1 and 14, MCV on chromosome 14, HEART on chromosomes 1 and 17, CARCASS on chromosome 9 and LIVER on chromosome 4) or fitted the data best (TBCC on chromosome 1, GIZZARD on chromosomes 2 and 6 and INTESTINE on chromosome 11). After fitting the relevant background genetic effects, for seven of these locations still the test statistic exceeded the suggestive genome-wide threshold (see tables 5.3 and 5.4), the model that included an imprinting effect fitted the data best, and the imprinting component was statistically different from zero (5% level). Except for the QTL for LNCREAT, the broiler allele coming through the male parent increased the trait value.

5.3.3.2 Results for QTL with sex-different effects

Results relating to searches for QTL with sex-different effects are shown in Table 5.5. As above, some locations showed suggestive linkage in the first stage of the study only when we allowed the QTL to have different effects across sexes.

We identified two significant and nine suggestive QTL with different effects in males and females (and in some cases mode of action) for both health-related traits and anatomical measures. As an example, a significant QTL on chromosome 13 for HEART acted in an overdominant fashion in males but only additively in females, with a smaller effect. For this QTL location the test statistic exceeded the 5% significance threshold also for a model with no sex interaction, but the fit of this model was significantly worse (5% level). This was also true for the QTL identified for LNTROP and GIZZARD.

For two of the suggestive QTL (LNCREAT on chromosome 1 and PCV on chromosome 2), including an imprinting component significantly improved the fit of the model. For both traits the estimate of the imprinting effect had opposite signs in males and females.

Two locations on sex chromosome Z showed suggestive linkage for LIVER and INTESTINE. In both cases the broiler allele increased the trait value, but to a larger extent for females than for males for LIVER and the other way round for INTESTINE. The dominance component was different from zero for both traits, which could be interpreted as a sign of interaction of the QTL alleles with the background of unrecombined broiler or layer Z chromosomes for males.

5.3.4 Two-QTL analyses

5.3.4.1 Results for QTL with sex-equal effects

Two-dimensional searches were carried out for all linkage groups with more than two markers. Single QTL were identified for CARCASS on both overlapping segments of chromosome 1. The length of segment 2 was modified so as to contain the QTL identified on segment 1 for the two dimensional search. Results are shown in Table 5.6.

After fitting unlinked QTL as cofactors, only for CARCASS on chromosome 1 did both the test statistic for two vs. no QTL and two vs. one QTL exceed the proposed thresholds for suggestive genome-wide linkage. The best locations corresponded to the QTL identified in the single QTL searches of the two segments in which chromosome 1 was originally divided to facilitate its analysis.

5.3.4.2 Results for QTL with sex-different effects

The analyses were repeated including a sex interaction, given that several QTL with different effects across sexes were identified in the single QTL searches. Results are shown in Table 5.7. A two-QTL model explained best the data for HEART on the second segment of chromosome 1. One of the locations corresponded to the QTL with sex-different effects identified when searching for a single QTL with sex-different effects.

Table 5.6. Results for a two QTL model with no sex interaction. Best locations from two-dimensional searches of linkage groups (LG) with more than two markers are presented together with test statistics (F ratio), estimates of QTL effects (and standard errors) at these locations, marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE).

Trait	LG	F ratio 4 df	F ratio 2 df	(1)	Location 1 (cM)	Location 2 (cM)	QTL1 Effects (se)		QTL2 Effects (se)		%VE (2)	Flanking markers QTL1		Flanking markers QTL2	
							a	*d*	*a*	*d*					
CARCASS	1	11.2	8.0	+	190	429	46.55 (11.60)	10.89 (21.51)	55.00 (11.44)	49.81 (18.30)	9.3	LEI0146	MCW0018	LEI0106	ADL0183

(1) + indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level respectively.
(2) Variance explained jointly by two linked QTL obtained as the percent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

Table 5.7. Results for a two QTL model with sex-different effects. Best locations from two-dimensional searches of linkage groups (LG) with more than two markers are presented together with test statistics (F ratio), estimates of QTL effects (and standard errors) at these locations, marker brackets for QTL locations and percentage of variance accounted for by the QTL (%VE).

Trait	LG	F ratio 8 df	F ratio 4 df	(1)	Location 1 (cM)	Location 2 (cM)	(2)	QTL1 Effects (se)		QTL2 Effects (se)		%VE (3)	Flanking markers QTL1		Flanking markers QTL2	
								a	*d*	*a*	*d*					
HEART	1	3.6	3.7	+	268	486	M	-0.14 (0.24)	2.01 (0.54)	-0.94 (0.24)	0.06 (0.48)	4.9	LEI0101	LEI0108	LEI0079	ROS0025
							F	0.01 (0.24)	-0.16 (0.52)	-0.03 (0.22)	-0.16 (0.48)					

(1) + indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level respectively.
(2) Sex: M is male and F female.
(3) Variance explained jointly by two linked QTL obtained as the percent reduction in residual mean squares after fitting the relevant fixed effects, covariates and cofactors.

5.3.5 *QTL effects*

The proportion of the phenotypic variance explained by the individual suggestive or significant QTL ranges from 2.0 (HEART) to 12.7% (CARCASS). By adding twice the additive effect estimated for all the suggestive or significant QTL, we estimated the overall effect of these QTL (*i.e.*, difference in trait values between broilers and layers accounted for by these QTL). Table 5.8 shows the overall effect for males, females and the mean of both expressed in absolute units and as a proportion of the F_2 population trait distribution residual standard deviation (F_2sd). As previously, a positive effect means that the broiler allele increases the trait value. The sign of the overall effects was the same for males and females, except for LIVER and LNTROP. Overall standardised effects (absolute values) ranged from 0.40 to 8.32 F_2sd for LIVER and CARCASS respectively in males and from 0.20 to 8.32 F_2sd for HEART and CARCASS in females. The overall effect for males expressed as a proportion of the effect for females ranged from -1.13 for LNTROP to 15.25 for HEART.

Table 5.8. Total trait difference explained by suggestive and significant QTL for males, females and the mean of both sexes expressed in trait units and in F_2 residual standard deviations (F_2sd). A positive difference means that broilers have a greater trait value.

Trait	Males		Females		Mean	
	Units	F_2sd (1)	Units	F_2sd	Units	F_2sd
LNCREAT (units)	-0.56	-1.44	-0.18	-0.46	-0.37	-0.95
LNTROP (units)	-0.18	-0.44	0.16	0.39	-0.01	-0.02
PCV (%)	1.52	0.89	1.26	0.74	1.39	0.82
TBCC (10^6/mm^3)	0.26	1.63	0.36	2.25	0.31	1.94
MCV (µm^3)	-4.06	-0.57	-4.06	-0.57	-4.06	-0.57
HEART (g)	-4.88	-3.07	-0.32	-0.20	-2.60	-1.64
CARCASS (g)	1328.64	8.32	1328.64	8.32	1328.64	8.32
LIVER (g)	-1.56	-0.40	2.32	0.59	0.38	0.10
SPLEEN (g)	-0.58	-0.73	-0.58	-0.73	-0.58	-0.73
GIZZARD (g)	-12.9	-2.86	-3.12	-0.69	-8.01	-1.78
INTESTINE (cm)	13.08	1.14	5.72	0.50	9.40	0.82

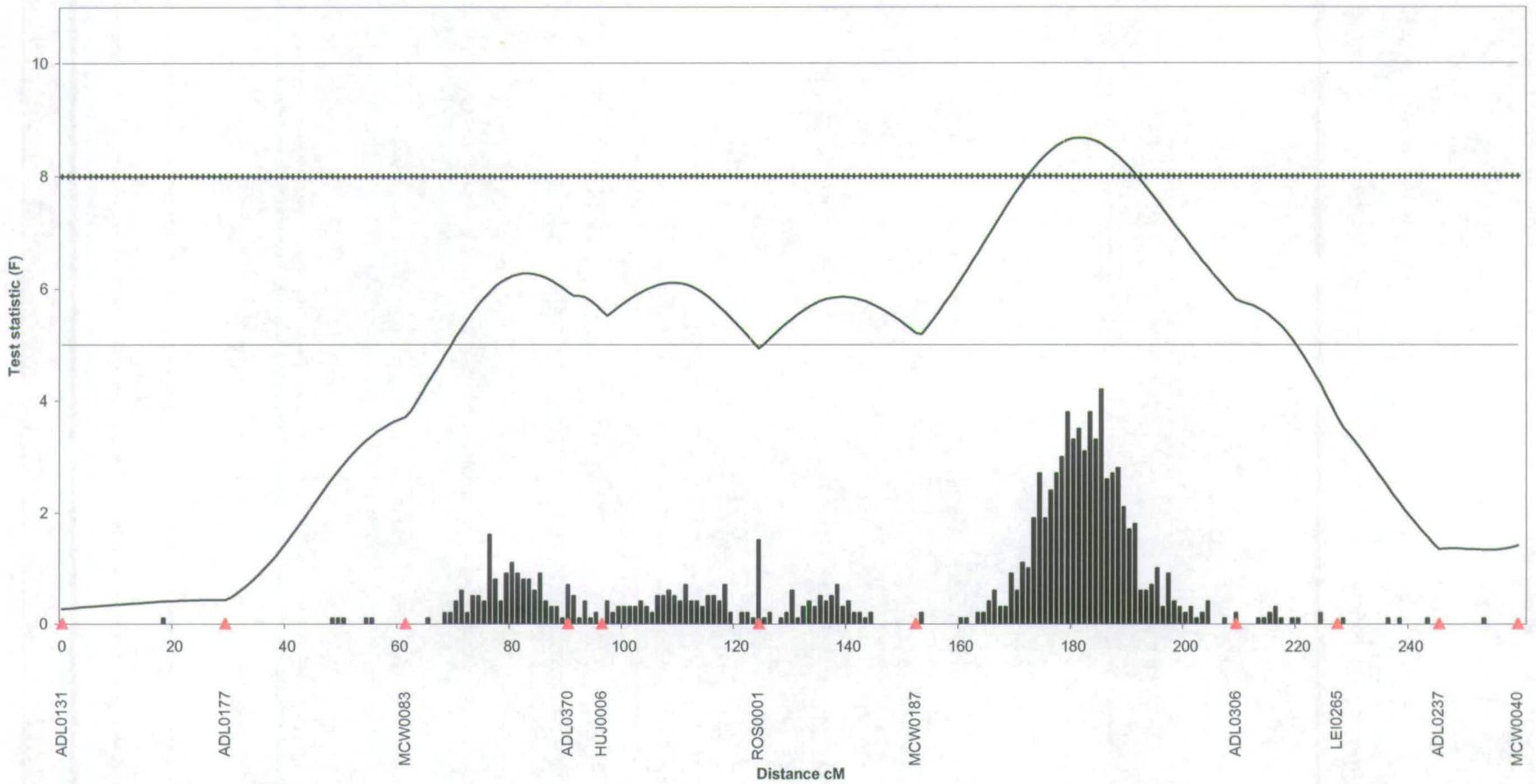
(1) Expressed in F_2 residual standard deviations.

5.3.6 *Confidence Intervals*

95% confidence intervals for QTL locations are presented on tables 5.4 and 5.5. These confidence intervals were generally large even for QTL that reached 5 or 1% genome-wide significance. In some cases they covered a large proportion of (or all) the length of the linkage group. Confidence intervals for QTL on chromosome 1 have been obtained for each segment, and could therefore be biased downwards.

Figure 5.1 shows the frequency distribution of the location parameter obtained by bootstrapping for CARCASS on chromosome 3. Especially in the case of long chromosomes, where several genetic factors may be affecting a given trait, the frequency distribution of the location can provide more information than the 95 % confidence interval.

Figure 5.1. F ratio profile (solid curve) and distribution of the QTL location parameter obtained by bootstrapping (histogram, 1000 resamples, arbitrary units) for CARCASS on chromosome 3. Locations for markers (red triangles) and marker names are also shown. Straight lines represent the genome-wide suggestive (solid line), 5 % (+) and 1% (-) thresholds.



5.3.7 *Searches for QTL with F_2 family interaction*

Only portions of linkage groups 1, 2, 3, 4, 5, 6, 9, 11, 13 and 28 could be scanned since, at given locations, markers were not informative within families. Table 5.9 shows the location with the highest test statistic for linkage groups with genome-wide suggestive or significant results, together with a comparison of the model with F_2 family interaction with a model with no interaction. No estimates for the QTL effects are given, since standard errors were very large given the small F_2 family sizes (6-27 individuals depending on families and traits). The test statistic exceeded the 5% genome-wide significance threshold for a model with F_2 family interaction for TBCC on one location on chromosome 1 and this model fitted the data better at this location than a model with a QTL with the same effect across families. For all other traits except LNTROP, MCV and INTESTINE the test statistic exceeded the genome wide suggestive threshold in one or more chromosomes. For 12 out of the 15 suggestive or significant locations, the model with family interaction fitted the data significantly better than a model with no interaction.

For locations where the test statistic for other models exceeded the relevant threshold, a model with family interaction was also compared to a model with a QTL with an additive and a dominance effect and no interaction (results not shown). This comparison was not always possible because a model that allowed for different effects across families could not be fitted for large portions of the genome. In these cases the model comparison was carried out few cM apart or was not carried out when it was not possible (for small linkage groups for example). Using this comparison, a model with a QTL with family-different effects did not fit the data best at any of the locations tested.

Table 5.9. Results for searches for QTL with different effects across F_2 families. The location with the highest test statistic for each linkage group (LG) with genome-wide suggestive or significant results are shown, together the with test statistics (F ratio), a comparison of fit of the model with F_2 family interaction with a model with no interaction (Yes/No and test statistic (Fit)) and the test statistic the model with no QTL x F_2 family interaction at these locations (F ratio $a + d$).

Trait	LG	F Ratio	(1)	Location (cM)	Fits best?	Fit	F ratio $a + d$
LNCREAT	1	1.6	+	335	Yes	1.7	0.4
PCV	1	1.6	+	335	Yes	1.5	5.8
PCV	5	1.6	+	66	Yes	1.6	1.0
PCV	9	1.7	+	5	Yes	1.7	0.8
TBCC	1	1.9	*	338	Yes	1.7	4.6
TBCC	2	1.6	+	348	No	1.3	10.6
TBCC	5	1.6	+	62	Yes	1.5	1.6
HEART	3	1.7	+	150	Yes	1.7	2.2
HEART	4	1.8	+	200	Yes	1.8	1.5
CARCASS	4	1.6	+	150	No	0.8	25.9
LIVER	6	1.6	+	19	Yes	1.6	2.9
SPLEEN	1	1.6	+	244	Yes	1.5	3.4
GIZZARD	2	1.6	+	146	No	1.3	12.1
GIZZARD	4	1.7	+	35	Yes	1.7	0.8
GIZZARD	9	1.6	+	95	Yes	1.6	1.0

(1) + indicates significance at the genome-wide suggestive level and * and ** at the 5% and 1% genome-wide level respectively.

5.4 Discussion

We have found strong support for QTL segregation for carcass and organ weights and blood parameters. Without taking into account the analyses that allowed for the interaction of the QTL with family, we have identified 11 genome-wide significant QTL (most of them for CARCASS) and several genome-wide suggestive ones.

We chose to scan the genome for four alternative QTL models, which increases the chances of obtaining false positives, compared to a more conservative strategy for which the genome would have been scanned once for a QTL with an additive and a dominance component and only the locations for which the test statistic exceeded the suggestive/significant genome-wide threshold would have been tested for alternative QTL models (with imprinting or sex interaction for example). In contrast, by using this more conservative strategy, one could potentially "miss" genuine QTL because the model used does not agree with the true genetic model (for example, a QTL with opposite effects in males and females would not be found). Scanning the genome only with the most complex models could also lead to one to "miss" QTL because of a lack of power (since in many cases we would be unnecessarily fitting extra parameters) (*e.g.* De Koning *et al.*, 2002).

All QTL significant at the genome-wide level would have been found by using the most conservative strategy but this would not have been the case for eight genome-wide suggestive QTL (for LNCREAT on chromosomes 1 and 9, HEART on chromosome 1, LIVER on chromosomes 4, TBCC on chromosome 11 and PCV on chromosomes 1 and 2).

Seldom the genome-wide significant QTL identified at the single QTL searches were picked up in the two-dimensional searches. This could be caused by the large number of degrees of freedom used in these tests.

Some of the suggestive QTL were amongst nine for which there was suggestive evidence of imprinting. There are few references suggesting the existence of imprinting in birds (see Koski *et al.* (2000) for an example). While we do not discard the existence of imprinting in chicken or that some of the traits studied might be affected by imprinted genes,

we are aware that these results might not be genuine but only statistical artefacts. By chance, combinations of linked (especially for long chromosomes) or unlinked genetic factors could create imprinting-like effects. De Koning *et al.* (2002) also showed by simulation that spurious detection of imprinting is a serious problem when dealing with QTL of small effect when founder lines are segregating, especially when few (F_1) parents of one sex are used. As an extreme example, one could think of a case where the QTL was segregating at the broiler line and, by chance, most of the male F_1 parents would have a given QTL genotype, different from the QTL genotype of most of the F_1 females. In this and less extreme scenarios, the variance explained by the QTL would be different across F_2 families and the data would be better explained if the QTL was allowed to have a different effect across families (or family types).

We carried out searches for single QTL with an additive and a dominance effect with F_2 family interaction, but a large proportion of the genome could not be scanned because of a lack of information in a number of families. Although no power study has been done, since the number of parameters to estimate is high when using a model that allows for different QTL effects across families, we can assume that power of this experiment to detect QTL segregating in the F_0 is low. Accordingly, one would expect to potentially "miss" even genuine QTL. Nonetheless, we observed a relatively high number of locations that showed evidence of suggestive or significant linkage. Some of these locations were close to already detected significant or suggestive QTL and, in some cases, the model with family interaction did not fit the data significantly best. Some other of the suggestive or significant locations found when searching for QTL with family-different effects were on linkage groups for which we had not previously found any evidence of linkage, or in linkage groups where we had, but at locations reasonably far away from the QTL identified assuming equal effects across families, suggesting that they were different. In these locations, generally, the test statistic for a model with no family interaction was low. For chromosomes where we had not previously detected QTL, we can suggest that these QTL are segregating within the F_0 lines

and hence the power to detect them when assuming that the QTL is fixed in the founder lines is low (especially if their effect is small) as demonstrated by simulation by Alfonso and Haley (1998). For linkage groups where other QTL had been identified in previous analyses, a simple explanation would be that there are two QTL on them, one (already detected in searches of single QTL with same effect across families) that would be fixed in the F_0 lines and a second one that is segregating. This is a plausible explanation since the locations identified with and without family interaction often correspond with the best locations of two-QTL analyses that failed to reach significance (results not shown). Alternatively, this could be an indication of a more complex genetic architecture of the traits: a simple model fails to detect QTL but, as the model becomes more complex, allowing for a difference in QTL effect depending on the parental origin of the allele (either imprinting or different effects across families) it can accommodate some of the "true" complications (or noise), and some locations become significant. In this respect, it is interesting to notice that -with the exception of chromosome 11- the suggestive imprinted QTL were located in either very long chromosomes (1 and 2) or chromosomes with poor marker coverage (4, 9 and 14). Both these scenarios would make it difficult to separate the effects of several genetic factors influencing a trait. In the latter case, improving this coverage would be beneficial.

The very long 95% confidence intervals obtained for some significant locations support the hypothesis of a complex genetic architecture. Including linked cofactors when estimating confidence intervals should decrease their length. This was indeed observed for confidence intervals on chromosome 1 (results not shown) but, in the cases where there is no clear evidence of the existence of more than one QTL on the linkage group, the choice of the locations to fit as cofactors may not be straightforward and it might be easier to simply refer to the frequency distribution obtained for the location parameter not fitting linked cofactors, since in these cases, inclusion of non-obvious cofactors could lead to biased results.

It is relevant to notice that when we searched the genome for QTL with different effects across families, suggestive or significant results arose more frequently for traits for

which the grandparental lines have not been heavily selected for (like organ weights and blood parameters) than for carcass weight, highly correlated with body weight, for which broilers have been intensely selected for decades. This long-term selection makes it more likely that grandparental lines are fixed for alternative CARCASS QTL alleles. Nonetheless, De Koning *et al.* (2003) detected QTL for growth in a pedigree of commercial broilers on chromosome 4. This study has been the first one to demonstrate that QTL identified in crosses of chicken populations were also segregating within a broiler population and the authors suggest that QTL for traits that have been long selected for could still be segregating because they may have pleiotropic effects on fitness traits.

In general, the results of our analysis are not conclusive for non-production traits in the sense that they do not provide a clear picture of the genetic control of the trait, but rather a series of hypothesis, that have not formally been tested against each other (which is not always possible). In any case, our results point at regions of the genome that it is worth exploring further.

Several authors (*e.g.* Lande and Thompson (1990), Utz *et al.* (2000), Göring *et al.* (2001)) have noted that the use of the same dataset to estimate QTL location and effect would lead to estimates of effects biased upwards, especially for QTL of small effect (because the bias depends on the power of the study). This has implications when it comes to experiment replication or use of findings in breeding programmes because not taking it into account would lead to an increased chance of failing to replicate the experiment or an overestimation of the gains to be accomplished with MAS or MAI programmes. Lande and Thompson (1990) suggested using independent sets of individuals to estimate markers to be included in a selection index and their additive effects (or equivalently QTL location and effect), but this is rarely done, as it would increase significantly the cost of the mapping experiments. As a less costly alternative, Utz *et al.* (2000) suggest the use of cross validation (a resampling method), but its implementation in the field of animal breeding or more markedly human genetics (Göring *et al.*, 2001) is difficult.

In our study, as it is common practice, the same data set was used to estimate QTL location and effect. It must therefore be borne in mind that the QTL effects presented are most likely overestimates of the true effects.

Although some of the signs of the estimated effects of some of the suggestive QTL are not in accordance with observed differences between layers and broilers (for example we have identified QTL for which the broiler allele decreases intestine length and increases relative heart weight), the significant QTL are in accordance with observations and so are in general the overall effects with the exception of LNCREAT. Our overall estimates would suggest that broilers would have lower creatinine kinase levels than layers. Individual QTL account for differences from ~ 40 (IU/l) to ~ 10 (IU/l), and estimates of additive effects are positive or negative. Although we do not have information on line differences for LNTROP, we would expect that overall broiler alleles would increase troponin T levels in blood, since the incidence of cardiomyopathies is higher in broilers, nonetheless only the estimated overall effect for females fits this expectation.

For LNCREAT we have found a suggestive location when searching for QTL with family interaction and the later model fitted the data better than one that assumes equal QTL effects across families. Estimates of QTL effects obtained assuming that the grandparental lines are fixed for alternative alleles are expected to be biased if this is not the case. The estimates obtained from the analyses with family interaction (not shown) are of opposite sign across families.

Overall QTL effects were different for males and females for some traits. Hocking *et al.* (1985) observed that adult outbred leghorn females had relatively heavier livers than heavy strain females but this was not the case in males. This observation is not in agreement with our results, but this might be due to the difference in age of the birds from both studies. Maxwell *et al.* (1990b) reported higher PCV in males than females and that fits with our findings. It has also been extensively reported that the incidence of ascites is higher in broiler

males than in females, and the extremely different effect across sexes for relative heart weight or LNTROP could provide an explanation for this.

Several mapping studies of growth related traits in chicken have been published recently. Sewalem *et al.* (2002) analysed the same population and found strong evidence of QTL for body weight on seven macrochromosomes (1, 2, 3, 4, 7, 8, and Z) and 2 microchromosomes (13 and 27) and suggestive evidence of linkage on chromosomes 5, 6 and 9. We found significant or suggestive QTL for carcass weight on chromosomes 1, 2, 3, 4, 8, 13 and 27. As expected, most of the QTL are located within the same marker bracket in both studies, given the high correlation of both traits. Van Kaam *et al.* (1999) found evidence for QTL for body weight at 48 days on chromosomes 1 and 4 in a population derived from a cross of broiler lines and Tatsuda and Fujinaka (2001) identified a QTL for weight at 16 weeks on chromosome 1. Li *et al.* (2002) studied a series of production traits, anatomical and physiological measures in two resource populations and identified associations of TGF-beta2 (located on chromosome 3) with spleen weight, tibia length, bone mineral content and density and blood content of glucagon, insulin, T3 and IGF2 in a resource population derived from a cross of a broiler and a Leghorn line. In the same population they found TGF-beta3 (located on chromosome 5) to be associated with body weight, percentage abdominal fat, spleen and liver weight, several bone characteristics and blood content of T3 and IGF2. In a different population (broiler x fayoumi cross) they found associations of TGF-beta3 with similar characters. We did not find evidence of linkage for SPLEEN with any location on chromosomes 3 or 5 or for LIVER or CARCASS on chromosome 5. Several authors (*e.g.* Luger *et al.* (2001)) have shown that broilers suffering from ascites also present al malfunction of the thyroidal axis and exhibit lower T3 and T4 levels. We have found evidence of genome-wide suggestive linkage for LNTROP with the region of chromosome 5 harbouring TGF-beta3 and therefore suggest that this region deserves further attention in metabolic disorders studies.

Rabie *et al.* (2002) described results from a whole genome scan for QTL affecting ascites-related traits carried out in a population derived from a cross of broiler lines. They reported three genome-wide significant QTL on chromosomes 2, 4 and 6 and suggestive QTL on chromosomes 1, 2, 3, 5, 8, 10, 13 and 28. This group has produced an F_8 derived from this population to validate and to narrow down the QTL regions. We picked up associations with locations on chromosomes 1, 2, 5, 6, 9, 11, 13, 14 and 17 for LNTROP, PCV, TBCC, MCV and HEART, which are all ascites related traits. The fact that QTL for relative heart weight have been found at different locations that relative weights of other organs suggests that heart QTL affect specifically relative heart weight rather than general differences in relative weight of organs between broilers and layers.

The number of birds showing clinical signs of ascites was very low in our F_2 population. It could be argued that, ascites incidence being very low in this population, birds considered outliers for LNTROP were indeed ascitic birds, since they all showed high LNTROP values. No data are available for us to verify this hypothesis. Analyses of LNTROP data including all phenotypic records were carried out (results not shown) and no evidence of linkage was found for chromosomes 5 or 11. In the other hand evidence of suggestive QTL on chromosomes 2 and 17 was robust to the inclusion of birds with extreme values in the analyses.

In the current study, we have identified QTL with moderate effects for ascites-related traits and an indicator of skeletal muscle damage and hypothesised that some of these QTL could be segregating within the founder lines. Phenotypic studies (see chapter three) and selection experiments (*e.g.* Druyan *et al.* (2001), Druyan *et al.* (2002), Wideman and French (1999) and Wideman and French (2000)) suggest that genes with large effect are involved in the control of ascites susceptibility and are segregating within broiler populations. Figure 5.2 shows locations of suggestive and significant QTL for each linkage group with more than two markers and more than one suggestive or significant QTL, for scans assuming equal QTL effects across F_2 families. Interestingly, associations with health-

related traits have been found in linkage groups that showed no association with carcass weight (a production trait). If QTL affecting ascites-related traits and muscle dysfunctions were segregating within broiler populations, manipulation of allele frequencies at these loci should allow improving broiler health without affecting carcass weight. It is difficult to throw any hypothesis in this respect for linkage groups that harbour both QTL for carcass weight and health-related traits given the large confidence intervals for the location parameter.

Given the welfare and economic consequences of muscular and metabolic disorders in commercial broiler populations, tools to reduce their incidence in broiler flocks would be welcome both by the industry and the consumer. Our findings are a first step towards the understanding of the genetic architecture of these complex disorders. We have pointed to some genome regions that seem to be associated with health-related traits. Further studies - with greater marker coverage, for instance- could provide the means to reduce the incidence of these disorders through MAS or MAI.

Figure 5.2. Locations of suggestive and significant QTL per linkage group (LG), for scans assuming equal QTL effects across F_2 families. Only autosomes with more than two markers and more than one suggestive or significant QTL are shown. Triangles (▲) represent marker locations and crosses (+) represent QTL locations. Genetic distances (cM) are also shown.

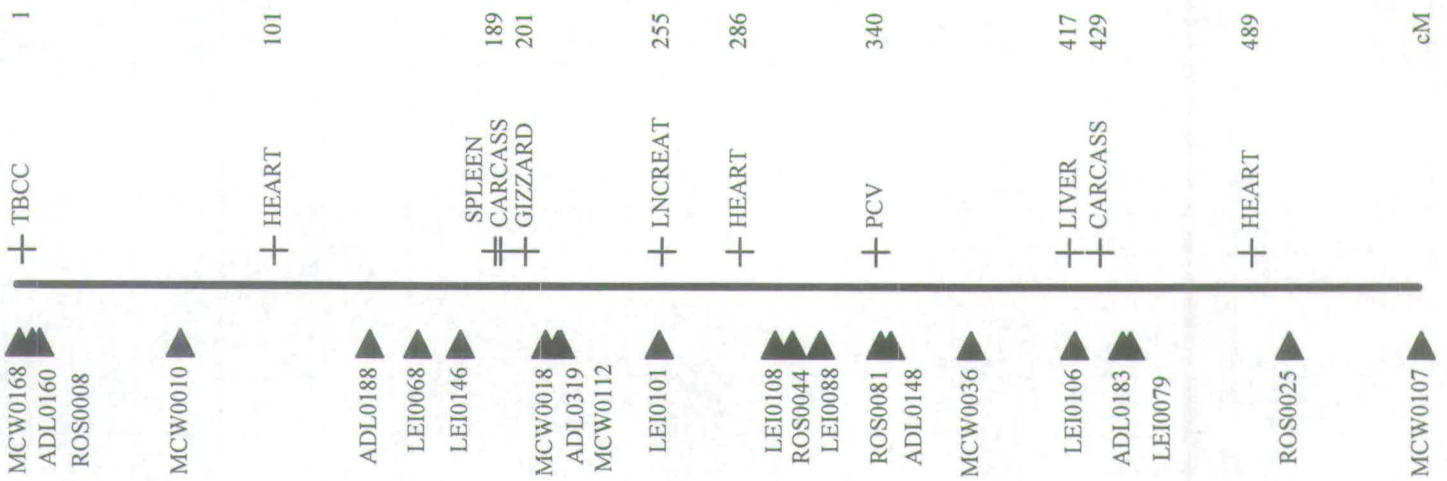
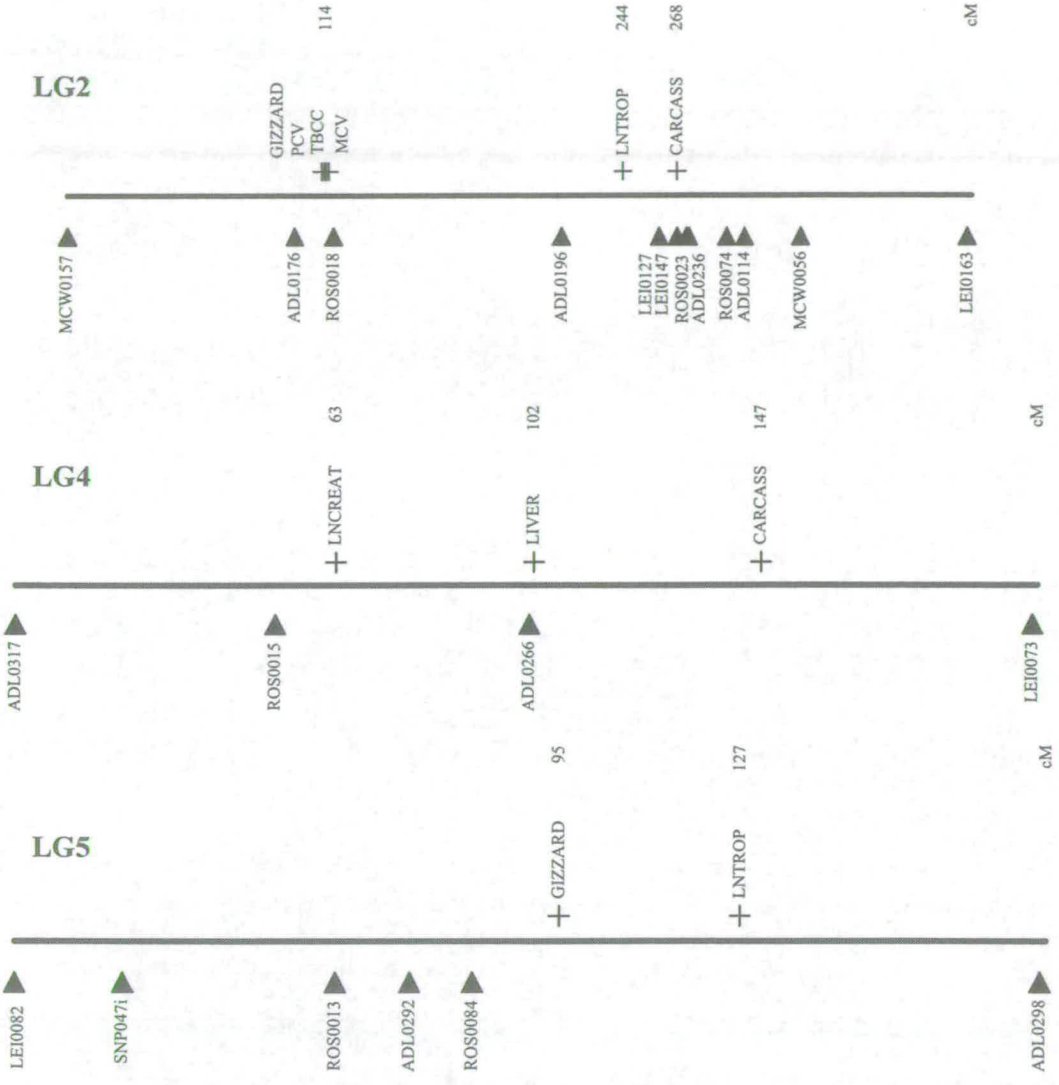


Figure 5.2 continued



CHAPTER SIX

6 GENERAL DISCUSSION

Ascites is a complex disorder with low incidence in well-managed flocks. It has been tacitly accepted that this metabolic disorder was, at least partially, under genetic control and somehow linked to the great improvement in growth and other production traits that has been accomplished in recent decades in meat-type chickens. We have demonstrated the existence of substantial genetic variation for SaO –an indicator of ascites susceptibility- in four meat-type chicken lines, and explored the genetic architecture of this trait in one of them. Specifically, we explored the hypothesis that loci with large effects were involved in the control of SaO and hence, potentially, ascites susceptibility. We analysed available pedigree and phenotypic data assuming that the trait was controlled by a putative single major locus and polygenes. Both the analyses of transformed and untransformed phenotypes suggested that a major locus was involved in the genetic control of SaO (although the allele frequencies, standardized gene effect size and mode of inheritance inferred by the two analyses differed). Following the results from segregation analyses on the untransformed scale, given that their interpretation was more straightforward, it was inferred that a dominant biallelic locus influenced SaO causing a difference between homozygous of around 13 % (*i.e.*, about 1.74 σ_p) in SaO levels. Taking into account only genotypes at this putative locus, ascitic birds would be the ones carrying two SaO decreasing alleles, and birds with other genotypes at this putative locus would show a lower incidence of the disorder. This locus was also estimated to have an overdominant effect on weight and fleshing score. An experimental population was created with paternal half-sib families from the breeding population. Families were chosen on the basis of the probability that the sire was segregating at the putative major locus affecting SaO levels, the ultimate aim of the experiment being to identify type I or type II markers linked to this putative locus. Our linkage study was focused on the regions around the three ryanodine receptors, and failed to detect significant

associations between SaO and these regions, although the marker information was limited for two of the regions so further study of these regions is needed.

In chapter two, we showed that there was substantial genetic variation for SaO in the four populations studied and therefore genetic selection on this trait was feasible. Moreover, we estimated that the genetic correlation between SaO and production traits was close to zero, suggesting that improvements on SaO could be achieved without compromising improvements in production. An estimated genetic correlation of zero does not mean, however, that there is no genetic relationship between the traits. There might be a genetic relationship, but this might not be additive. De Greef *et al.* (2001a) pointed out how complex interactions between traits could hinder estimation of genetic correlations. It has been suggested that birds that are genetically capable of rapid and high growth, and are reared in an environment that favours the expression of this growth potential, have a high oxygen demand that cannot be fulfilled. This triggers the presence of ascites and prevents further expression of their growth potential. Genotypes that have a lower growth genetic potential, even if they are equally genetically prone to suffer from ascites, would be less likely to develop the disorder and therefore their growth would not be compromised. Under this model, the estimated genetic correlation in a population would depend on the incidence of the disorder and would be a function of the correlation estimated from the ascitic and non-ascitic subpopulations. This would not only make difficult to interpret the estimated correlations, but would also compromise their use in multitrait selection, since the outcome of selection may not be as predicted by the simple additive model. This would be especially so if estimates were obtained from a population somehow challenged to increase the incidence of the disorder.

In chapter three, we presented evidence that a dominant locus with large effect on SaO levels was segregating in line 3 and that this locus also affected weight and fleshing score in an overdominant fashion. The work of Pavlidis *et al.* (2003), who observed significant positive heterosis for body weight in crosses of ascites-resistant and ascites-

susceptible lines supports our inference about the mode of action of the putative locus in production traits. The mode of action of such locus on SaO and production would explain why a locus with negative effects on a fitness (health and welfare) trait would be segregating in the population at intermediate frequencies. A similar mechanism was responsible of leading to a balanced polymorphism at the halothane sensitivity locus in pigs (Smith, 1982). In this case, while the susceptibility allele had negative effects on stress susceptibility, it also had positive effects on carcass traits (leanness). The equilibrium frequencies at which the alleles were maintained within each breed depended on the amount of selection on lean and stress susceptibility. In cattle, the myostatin gene, that has antagonistic effects on muscling score and calving ease, provides another example of a polymorphism with negative effect on fitness that is maintained given its positive effect on production.

There are few examples of proven overdominance in animals for fitness or production traits. Falconer and Mackay (1996) refer to sickle-cell anaemia in humans and warfarin resistance in rats and, more recently, Cockett *et al.* (1996) reported polar overdominance at the callipyge locus in sheep. Falconer and Mackay (1996) also discuss how overdominance or pseudo-overdominace can arise. We hypothesise that the above-described interdependence between growth/production and ascites could lead to the inference that the putative locus that affects SaO has an overdominant effect on growth/production when in fact its direct action on production traits might be, for example, just additive. Table 6.1 shows the genetic values at the putative locus for SaO and production together with the expected phenotypic values, assuming a dominant mode of action of the locus for SaO and an additive mode of action for production and assuming that expression of both ascites and production is interdependent.

Table 6.1. Genetic values at the putative locus for SaO and growth together with the expected phenotypic values, assuming a dominant mode of action of the locus for SaO and an additive mode of action on growth and assuming that expression of both ascites and production is interdependent.

	Major Genotype	*BB*	*Bb*	*bb*
Genetic value	**SaO**	High	High	Low
	Production	Low	Intermediate	High
Phenotypic value	**Ascites**	Resistant	Resistant	Susceptible/Affected
	Production	Low	Highest	Deprived/Low

If major allele *b* has direct positive effects on production but negative effects on ascites resistance then, in the presence of (clinical or subclinical) ascites it will have indirect negative effects on production and *bb* birds will perform poorly for both traits. *Bb* heterozygotes would be ascites resistant with better production performance than affected *bb* homozygotes, whilst *BB* birds would be healthy but have a poorer production performance than *Bb* heterozygotes. This advantage in "performance achieved" would mean that the major genotype *Bb* would have a selection advantage over either homozygote, making the elimination of the "low SaO allele" (*i.e.*, *b*) from the population a difficult task without the use of molecular markers. Under this model, selection directly against low SaO using oximeter measurements would shift the balance a bit, but the *Bb* heterozygote would have a selective advantage over either homozygote, so the deleterious *b* allele would be retained in the population.

In chapter three, we also pointed out that segregation analysis is not robust to deviations from normality not caused by segregation of major locus and that this could lead to the detection of spurious loci. The work of Janss and collaborators (1997a, 1997b) and De Koning and collaborators (see De Koning, 2001) -discussed in chapter three- provides an example of putative loci identified by segregation analyses that could not be confirmed in a mapping study. On a more positive note, Le Roy *et al.* (1990) identified a major dominant locus affecting "Napole yield" (RN) in pigs by segregation analysis. In 1995, Milan *et al.* (1995) identified in a linkage study a QTL on pig chromosome 15 that corresponded to the

major locus identified by Le Roy *et al.* (1990) and Milan *et al.* (2000) identified a causative mutation in the PRKAG3 gene. We are aware that segregation analyses results need to be taken with caution, but the results we obtained from these analyses are consistent amongst them and with complementary analyses carried out within this thesis. For instance, a simulation study showed that if a dominant major locus that affected SaO were present in a population undergoing selection for increased SaO and the gene effect and allele frequencies were as inferred by segregation analysis, parameter estimates obtained from analyses assuming a purely polygenic model and analyses assuming a mixed inheritance model would be consistent. This simulation study and analyses of subsets of birds classified as "ascitic" and "non-ascitic" on the basis of their SaO record further reinforce segregation analyses results. Specifically, when analysed assuming a purely polygenic model, subsets that included proportions of birds that were in close agreement with the proportions of "low SaO" / "ascitic" and "high SaO" / "non-ascitic" birds predicted from segregation analyses on the basis of the genotype at the putative major locus, yielded estimates of heritability for SaO smaller than the one obtained from the whole data set and of a magnitude that would be compatible with of the estimated within major-genotype (*i.e.*, polygenic) heritability. In addition, independent work by Druyan and collaborators (2001, 2002) and Wideman and French (1999,2000) points in the same direction as our own: a major locus affects ascites susceptibility and segregates within broiler populations. Furthermore, work by Pavlidis and collaborators (2003) supports the inferred mode of action of the putative gene on production traits, since they demonstrated that crosses of ascites-resistant and ascites-susceptible lines outperformed the pure lines they originated from for several production traits, amongst them weight. These groups studied stock from sources other than our own. Eitan and Soller (2002) noticed how, remarkably, deleterious effects on broiler health and reproduction seem to appear simultaneously in the stocks of all main breeders. This could be due to informal entries of immigrants into selection nuclei, which would make that mutations arising in a population would be rapidly spread into others. On the other hand, Eitan and Soller (2002)

suggested that selection –for similar traits and with similar pressure- could affect homeostasis or genetic background similarly in different population and this, in turn, would make that new sets of genes "become active" simultaneously in all populations.

We designed a resource population (a collection of 20 paternal half-sib families) to map the putative locus identified in chapter three. For our linkage study, we chose to focus on regions around the three ryanodine receptors (RYRs), that were judged to be strong candidates implicated in the control of ascites susceptibility, since ryanodine receptor 2 had been shown to lie within a region affecting pulmonary hypertension in rats and several cardiomiopathies in humans. No strong evidence of linkage of SaO with any of the regions studied was found but, as we discussed in chapter four, further work is necessary to rule out any implication of Ryanodine receptors 1 and 2 on the genetic control of SaO. If this work still failed to produce positive results, the resource population created would allow testing other candidate loci, as discussed in chapter four or alternatively, a genome scan could be carried out. In chapter five, we identified regions affecting ascites-related traits in a broiler x layer cross. These regions could also be used in further studies in our collection of half-sib families, since QTL responsible for between-breed variation could also be implicated in the control of ascites within broiler populations.

The linkage mapping study provided strong evidence of a QTL for fleshing score within the ryanodine receptor 3 linkage group. This finding certainly merits further attention. Markers linked to this QTL could be used in MAS within this population. To this aim, the segregation of this putative QTL in the population would need to be confirmed and a thorough study of its pleiotropic effects on other traits would be necessary. In addition, regions flanking the markers used in our analysis would need to be explored since the QTL detected could well lie outside the marker interval studied.

Marker assisted selection (MAS) has been shown to produce additional response when applied in an outbred poultry breeding population (Van der Beek and Van Arendonk, 1996). The use of MAS in traits that are not difficult to measure, nor sex-limited and respond

well to traditional selection is nonetheless questionable, given its relatively high cost, and this is more so in the poultry industry, where the generation interval is short and the value of individual birds is relatively low. This technology would nonetheless be of great value in traits such as ascites susceptibility where there is strong evidence for the involvement of a major locus in the control of the trait. Even though easy-to-measure traits such as SaO are reliable indicators of susceptibility to the disorder and heritable, which makes selection against the disorder feasible, the potentially complex relationship between production and disorder-related traits would make the use of genetic markers the most efficient means to manage the frequency at this locus in multitrait selection programmes.

The study of other regions that are likely to affect SaO or other ascites-related traits could lead to the identification of genetic markers linked to ascites susceptibility. As previously mentioned, further studies could initially focus on a series of candidate regions. These could be regions suggested by physiological or comparative evidence, or by gene expression studies but regions inferred from line-cross experiments merit also consideration. Given the wealth of candidate regions that could potentially be tested, a genome scan of the population of interest could be considered.

Assuming that a gene was responsible for a large proportion of the variation in SaO levels (or other ascites-related traits) and hence potentially affected ascites susceptibility, ideally this gene would be identified and one point mutation responsible for the "ascites-susceptibility" allele would be detected. This would allow producing a direct DNA test that would enable us to fully control allele frequency at this locus. In livestock populations, the identification of point mutations responsible for Halothane sensitivity in pigs (Fujii *et al.*, 1991) and LAD in cattle (Shuster *et al.*, 1992) have allowed the development of such diagnostic tests, that are used in commercial breeding and that can be used across populations. The identification of mutations that are directly responsible for a given phenotype requires, nonetheless, not only a large amount of work but also a fair amount of luck. Once regions linked to SaO or other ascites-related traits have been identified (for

example in a linkage study as presented in chapter four) and confirmed in the population of interest, further studies – with denser marker maps- may be necessary to refine the location of the QTL so that the confidence interval of this parameter is around or under 5 cM. This could be done through genetic chromosome dissection (see Darvasi, 1998), where progeny of sires heterozygous at the QTL (with known haplotypes at the QTL region) would be screened for recombinations within the QTL region and progeny-tested to determine the QTL status of the recombinant chromosome. Once the confidence interval of the QTL location has been reduced, it would be possible to further increase marker density and use linkage disequilibrium mapping methods within this reduced chromosomal region to identify polymorphism(s) that affect the trait studied. These polymorphisms could either be causative of the phenotype of interest or be tightly linked to it, which would also allow the development of genetic (diagnostic) tests. If the locus were not causative, associations would need to be re-evaluated for every population studied and over time, since recombination may occur between the "diagnostic marker" and the causative locus. Alternatively, candidate genes could be identified within the reduced chromosomal region, and sequences from individuals with extreme phenotypes compared, in the hope that sequence differences reveal a causative mutation (validation in a wider population would then be needed). This approach could also be implemented if gene expression studies revealed differences in gene expression between extreme phenotypes. These strategies are costly and time consuming, but could ultimately produce tools for allele frequency manipulation that could be used directly across populations and generations (when the causative mutation is identified) or with little routine re-evaluation (when only associated –but not causative- polymorphisms are identified). In some circumstances, when there is evidence of the existence of a QTL segregating in a population, it might be desirable to directly use information from linked markers in assisted breeding without knowledge of causative or tightly linked polymorphism. This would allow the use of valuable information within a much shorter time-span. To this aim within-family marker-trait associations can be used, but need to be continuously re-evaluated.

Our work has set up the basis for further investigations of the genetics of ascites. Given the consistency of results presented in this thesis and their agreement with results of independent studies, pursuing the work we have initiated is worthwhile. Given the effect of the putative locus on SaO and production, molecular markers would provide the most efficient means of controlling allele frequencies at this locus within commercial breeding programmes and would allow reducing to a minimum the incidence of the disorder, or even of creating a fully ascites resistant population.

REFERENCES

Airey JA, Baring MD, Beck CF, Chelliah Y, Deerinck TJ, Ellisman MH, Houenou LJ, Mckemy DD, Sutko JL & Talvenheimo J (1993). Failure to make normal alpha-ryanodine receptor is an early event associated with the crooked neck dwarf (cn) mutation in chicken. Developmental Dynamics, 197: 169-188.

Alfonso L & Haley CS (1998). Power of different F-2 schemes for QTL detection in livestock. Animal Science, 66: 1-8.

Arai M, Matsui H & Periasamy M (1994). Sarcoplasmic-reticulum gene-expression in cardiac-hypertrophy and heart-failure. Circulation Research, 74: 555-564.

Bauce B, Rampazzo A, Basso C, Bagattin A, Daliento L, Tiso N, Turrini P, Thiene G, Danieli GA & Nava A (2002). Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death early diagnosis of asymptomatic carriers - Early diagnosis of asymptomatic carriers. Journal Of The American College Of Cardiology, 40: 341-349.

Beilharz RG, Luxford BG & Wilkinson JL (1993). Quantitative genetics and evolution - is our understanding of genetics sufficient to explain evolution. Journal Of Animal Breeding And Genetics-Zeitschrift Fur Tierzuchtung Und Zuchtungsbiologie, 110: 161-170.

Center for Devices and Radiological Health (CDRH), 1997. URL: www.fda.gov/cdrh

Chapman HD, Johnson ZB & McFarland JL (2003). Improvements in the performance of commercial broilers in the USA: analysis for the years 1997 to 2001. Poultry Science, 82: 50-53.

Cheema MA, Qureshi MA & Havenstein GB (2003). A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poultry Science (accepted).

Classen HL (2000). Managing metabolic disease in rapidly growing strains of poultry, in BSAS occasional publication n° 27: The challenge of genetic change in animal production, pp. 63-64.

Cockett NE, Jackson SP, Shay TL, Farnir F, Berghmans S, Snowder GD, Nielsen DM & Georges M (1996). Polar overdominance at the Ovine callipyge locus. Science, 273: 236-238.

Cockett NE, Jackson SP, Shay TL, Nielsen D, Moore SS, Steele MR, Barendse W, Green RD & Georges M (1994). Chromosomal localization of the callipyge gene in sheep (ovis-aries) using bovine dna markers. Proceedings Of The National Academy Of Sciences Of The United States Of America, 91: 3019-3023.

Currie, RJW (1999). Ascites in poultry: recent investigations. Avian Pathology, 28: 313-326.

Darvasi A (1998). Experimental strategies for the genetic dissection of complex traits in animal models. Nature Genetics, 18: 19-24.

Darvasi A & Soller M (1992). Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theoretical and Applied Genetics, 85: 353-359.

Decuypere E, Buyse J & Buys N (2000). Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. World's Poultry Science Journal, 56: 367-377.

Deeb N & Lamont SJ (2002). Genetic architecture of growth and body composition in unique chicken populations. Journal Of Heredity, 93: 107-118.

De Greef KH, Janss LLG, Vereijken ALJ, Pit R & Gerritsen CLM (2001a). Disease-induced variability of genetic correlations: Ascites as a case study. Journal of Animal Science, 79: 1723-1733.

De Greef KH, Kwakernaak C, Ducro BJ, Pit R & Gerritsen CLM (2001b). Evaluation of between-line variation for within-line selection against ascites in broilers. Poultry Science, 80: 13-21.

De Koning DJ (2001). Identification of (non-) Mendelian factors affecting pork production. PhD Thesis, Wageningen University.

De Koning DJ, Bovenhuis H & Van Arendonk JAM (2002). On the Detection of Imprinted Quantitative Trait Loci in Experimental Crosses of Outbred Species. Genetics, 161: 931-938.

De Koning DJ, Windsor D, Hocking PM, Burt DW, Law A, Haley CS, Morris A, Vincent J, Griffin H. (2003). QTL detection in commercial broiler lines using candidate regions. Journal of Animal Science, 81: 1158-1165.

Demenais F, Lathrop M & Lalouel JM (1986). Robustness and power of the unified model in the analysis of quantitative measurements. American Journal of Human Genetics, 38: 228-234.

Druyan S, Ben-David A, Bellaiche M, Rosner A, Shlosberg A & Cahaner A (2001). Genetic differences in the susceptibility of broilers to ascites. Proceedings of the 2nd European Poultry Genetics Symposium, Gödöllő (Hungary) pp. 85-86.

Druyan S, Ben-David A, Bellaiche M, Shlosberg A, Yahav S & Cahaner A (2002). Indications for a major gene for broiler susceptibility to ascites. Proceedings of the 11th European Poultry Conference, Bremen (Germany) p. 300. CD-ROM communication.

Druyan S, Cahaner A, Bellaiche M, Rosner A & Shlosberg A (1999). Genetic evaluation of blood oxygenation, heart rate, electrocardiographic (ECG) waveforms, and their association with ascites in broilers. Presented at the 1999 European Poultry Breeders Roundtable, Wiesensee (Germany).

Dunner S, Miranda ME, Amigues Y, Canon J, Georges M, Hanset R, Williams J & Menissier F (2003). Haplotype diversity of the myostatin gene among beef cattle breeds. Genetics Selection Evolution, 35: 103-118.

Dunnington EA & Siegel PB (1996). Long-term divergent selection for eight-week body weight in White Plymouth Rock chickens. Poultry Science, 75: 1168-1179.

Eitan Y & Soller M (2002). Associated effects of sixty years of commercial selection for juvenile growth rate in broiler chickens : endo/exophysiological, or genetic? 7th World Congress on Genetics Applied to Livestock Production, Montpellier (France). CD-ROM communication n° 19-10.

Elston RC & Stewart J (1971). A general model for the genetic analysis of pedigree data. Human Heredity, 21: 523-542.

Emmans GC & Kyriazakis I (2000). Issues arising from genetic selection and body composition characteristics in poultry and pigs, in BSAS occasional publication n° 27: The challenge of genetic change in animal production, pp. 39-52.

Emmerson DA (1997). Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. Poultry Science 76: 1121-1125.

Fairfull RW, McMillan L & Muir WM (1998). Poultry breeding: progress and prospects for genetic improvement of egg and meat production. 6th World Congress on Genetics Applied to Livestock Production, Armidale (Australia).

Falconer DS & Mackay TFC (1996). Introduction to quantitative genetics. 4th edition. Longman, Harlow.

Fatkin D & Graham RM (2002). Molecular mechanisms of inherited cardiomyopathies. Physiological Reviews, 82: 945-980.

Fisher RA (1918). The correlation between relatives under the supposition of Mendelian inheritance. Transactions of the Royal Society of Edinburgh, 52: 399-433.

Fujii J, Otsu K, Zorzato F, Deleon S, Khanna VK, Weiler JE, Obrien PJ & Maclennan DH (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253: 448-451.

GENSTAT 5 COMMITTEE (1993). GenstatTM 5 release 3 reference manual. Clarendon Press,Oxford, UK.

Geyer CJ (1992). Practical Markov chain Monte Carlo. Statistical Science, 7: 473-511.

Gilmour AR, Cullis BR, Welham SJ & Thompson R (2000). ASREML Reference Manual 2nd edition, Release 1.0 NSW Agriculture Biometrical Bulletin 3, NSW Agriculture, Locked Bag, Orange, NSW 2800, Australia. ftp://ftp.res.bbsrc.ac.uk/pub/aar/

Göring HHH, Terwilliger JD & Blangero J (2001). Large upward bias in estimation of locus-specific effects from genomewide scans. American Journal of Human Genetics 69:1357-1369.

Green P, Falls K & Crooks S (1990). Cri-Map version 2.4. Washington University School of Medicine, St. Louis.

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M & Snell R (2002). Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Research, 12: 222-231.

Groenen MAM, Cheng HH, Bumstead N, Benkel BF, Briles WE, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, de Leon AP, Soller M, Takahashi H & Vignal A (2000). A consensus linkage map of the chicken genome. Genome Research, 10: 137-147.

Gu DF, O'Dell SD, Chen XH, Miller GJ & Day INM (2002). Evidence of multiple causal sites affecting weight in the IGF2-INS-TH region of human chromosome 11. Human Genetics, 110: 173-181.

Guernec A, Berri C, Chevalier B, Wacrenier-Cere N, Le Bihan-Duval E & Duclos MJ (2003). Muscle development, insulin-like growth factor-I and myostatin mRNA

levels in chickens selected for increased breast muscle yield. Growth Hormone & Igf Research, 13: 8-18.

Guo SW & Thompson EA (1991). Monte-Carlo estimation of variance component models for large complex pedigrees. IMA Journal Of Mathematics Applied In Medicine and Biology, 8: 171-189.

Guo SW & Thompson EA (1994). Monte-Carlo estimation of mixed models for large complex pedigrees. Biometrics, 50: 417-432.

Haley CS, Knott SA & Elsen JM (1994). Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics, 136: 1195-1207.

Haley CS, Lee GJ & Ritchie M (1995). Comparative reproductive-performance in Meishan and Large White pigs and their crosses. Animal Science, 60: 259-267.

Hanset R & Michaux C (1985). On the genetic determinism of muscular hypertrophy in the belgian white and blue cattle breed .1. Experimental-data. Genetics Selection Evolution, 17: 359-368.

Hanset R & Michaux C (1985). On the genetic determinism of muscular hypertrophy in the belgian white and blue cattle breed .2. Population-data. Genetics Selection Evolution, 17: 369-385.

Havenstein GB, Ferket PR & Qureshi MA (2003a). Carcass composition and yield of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science (accepted).

Havenstein GB, Ferket PR & Qureshi MA (2003b). Growth, livability, and feed conversion of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science (accepted).

Havenstein GB, Ferket PR, Scheideler SE & Rives DV (1994). Carcass composition and yield of 1991 vs 1957 broilers when fed "typical" 1957 and 1991 broiler diets. Poultry Science, 73: 1795-1804.

Hill WG (1984). On selection among groups with heterogeneous variance. Animal Production, 39: 473-477.

Hill WG (1996). Mutations and selection response in quantitative traits. Proceedings of the National Breeder's Roundtable Meeting. St. Louis, Missouri.

Hill WG (2002). Direct effects of selection on phenotypic variability of quantitative traits. 7th World Congress on Genetics Applied to Livestock Production, Montpellier (France). CD-ROM communication n° 19-02.

Hocking PM, Gavora JS, Chambers JR & Fortin A (1985). Genetic-variation in body size, composition, temperature, and feed-intake in mature chickens. Poultry Science, 64: 6-28.

Hunton P (1998). The potential of genetics to combat ascites. World Poultry, 14: 64-66.

Ikeobi CON, Woolliams JA, Morrice DR, Law A, Windsor D, Burt DW & Hocking PM (2002). Quantitative trait loci affecting fatness in the chicken. Animal Genetics, 33: 428-435.

Jansen R (1993). Interval mapping of multiple quantitative trait loci. Genetics, 135: 205-211.

Janss LLG, Thompson R & Van Arendonk JAM (1995). Application of Gibbs sampling for inference in a mixed major gene-polygenic inheritance model in animal populations. Theoretical and Applied Genetics, 91: 1137-1147.

Janss LLG, Van Arendonk JAM & Brascamp EW (1997a). Bayesian statistical analyses for presence of single genes affecting meat quality traits a crossed pig population. Genetics, 145: 395-408.

Janss LLG, Van Arendonk JAM & Brascamp EW (1997b). Segregation analyses for presence of major genes affecting growth, backfat, and litter size in Dutch Meishan crossbreds. Journal of Animal Science, 75: 2864-2876.

Jensen P & Barton-Gade P (1985). Performance and carcass characteristics of pigs with known genotypes for halotane susceptibility. In: Stress susceptibility and meat quality in pigs. European Association of Animal Production Publications pp. 33-80.

Julian RJ (1993). Ascites in poultry. Avian Pathology, 22: 419-454. 1993.

Julian RJ & Mirsalimi SM (1992). Blood-oxygen concentration of fast-growing and slow-growing broiler-chickens, and chickens with ascites from right ventricular failure. Avian Diseases, 36: 730-732.

Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N & Groffen J (1995). Abnormal lung development and cleft-palate in mice lacking TGF-beta-3 indicates defects of epithelial-mesenchymal interaction. Nature Genetics, 11: 415-421.

Knott SA, Elsen JM & Haley CS (1996). Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theoretical and Applied Genetics, 93: 71-80.

Knott SA, Haley CS & Thompson R (1992a). Methods of segregation analysis for animal breeding data: a comparison of power. Heredity, 68: 299-311.

Knott SA, Haley CS & Thompson R (1992b). Methods of segregation analysis for animal breeding data: parameter estimates. Heredity, 68: 313-320.

Knott SA, Marklund L, Haley CS, Andersson K, Davies W, Ellegren H, Fredholm M, Hansson I, Hoyheim B, Lundstrom K, Moller M & Andersson L (1998). Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and Large White pigs. Genetics, 149: 1069-1080.

Koerhuis ANM & Thompson R (1997). Models to estimate maternal effects for juvenile body weight in broiler chickens. Genetics Selection Evolution, 29: 225-249.

Koski LB, Sasaki E, Roberts RD, Gibson J & Etches RJ (2000). Monoalleleic transcription of the insulin-like growth factor-II gene (Igf2) in chick embryos. Molecular Reproduction and Development, 56:345-352.

Laitinen PJ, Brown KM, Piippo K, Swam H, Devaney JM, Brahmbhatt B, Donarum EA, Marino M, Tiso N, Viitasalo M, Toivonen L, Stephan DA & Kontula K (2001). Mutations of the cardiac ryanodine receptor (RYR2) gene in familial polymorphic ventricular tachycardia. Circulation, 103: 485-490.

Lalouel JM, Rao DM, Morton NE & Elston RC (1983). A unified model for complex segregation analysis. American Journal of Human Genetics, 35: 816-826.

Lande R & Thompson R (1990). Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics, 124: 743-756.

Lander E & Kruglyak L (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics, 11: 241-247.

Langley B, Thomas M, Bishop A, Sharma M, Gilmour S & Kambadur R (2002). Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. Journal Of Biological Chemistry, 277: 49831-49840.

Law AS & Archibald AL (2000). Farm animal genome databases. Briefings in Bioinformatics, 1: 151-160.

Leroy P & Elsen JM (1992). Simple test statistics for major gene detection: a numerical comparison. Theoretical and Applied Genetics, 83: 635-644.

Leroy P, Naveau J, Elsen JM & Sellier P (1990). Evidence for a new major gene influencing meat quality in pigs. Genetical Research, 55: 33-40.

Li H, Deeb N, Zhou H, Ashwell CM & Lamont SJ (2002). Chicken QTLs for growth, body composition, and metabolic factors associated with TGF-BETA family genes. Abstract of a poster presented at the Plant, Animal and microbe Genomes X Conference.

Li H, Deeb N, Zhou H, Mitchell AD, Ashwell CM & Lamont SJ (2003). Chicken quantitative trait loci for growth and body composition associated with transforming growth factor-beta genes. Poultry Science, 82: 347-356.

Lubritz DL, Smith JL & McPherson BN (1995). Heritability of ascites and the ratio of right to total ventricle weight in broiler breeder male lines. Poultry Science, 74: 1237-1241.

Luger D, Shinder D, Rzepakovsky V, Rusal M & Yahav S (2001). Association between weight gain, blood parameters, and thyroid hormones and the development of ascites syndrome in broiler chickens. Poultry Science, 80: 965-971.

Lui WY, Wong CH, Mruk DD & Cheng CY (2003). TGF-beta 3 regulates the blood-testis barrier dynamics via the p38 mitogen activated protein (MAP) kinase pathway: An in vivo study. Endocrinology, 144 : 1139-1142.

MacLean CJ, Morton NE & Lew R (1975). Analysis of family resemblance. IV. Operational characteristics of segregation analysis. American Journal of Human Genetics, 27: 365-384.

Maclean CJ, Morton NE, Elston RC & Yee S (1976). Skewness in commingled distributions. Biometrics, 32: 695-699.

Maxwell MH & Robertson GW (2000). Cardiovascular disease in poultry: Epidemiology – Current trends and correlates. Proceedings of the Annual Meeting of the Poultry Science Association, August 18-21, Montreal. http://www.poultryscience.org/abs00/00psab25.pdf

Maxwell MH & Robertson GW (1997). World broiler ascites survey 1996. Poultry International, April: 16-30.

Maxwell MH, Robertson GW, Bautista-Ortega J & Hocking PM (1998). A preliminary estimate of the heritability of plasma troponin T in broiler chickens. British Poultry Science, 39: 16-19.

Maxwell MH, Robertson GW & Moseley D (1994). Potential role of serum troponin-T in cardiomyocite injury in the broiler-ascites-syndrome. British Poultry Science, 35: 663-667.

Maxwell MH, Robertson GW & Moseley D (1995). Serum troponin t values in 7-day-old hypoxia-treated and hyperoxia-treated, and 10-day-old ascitic and debilitated, commercial broiler chicks. Avian Pathology, 24: 333-346.

Maxwell MH, Robertson GW & Spence S (1986). Studies on an ascitic syndrome in young broilers 1. Haematology and pathology. Avian Pathology, 15: 511-524.

Maxwell MH, Robertson GW, Spence S & McCorquadale CC (1990a). Comparison of haematological values in restricted and ad-libitum fed domestic fowls: red blood cell characteristics. British Poultry Science, 31: 407-413.

Maxwell MH, Spence S, Robertson GW & Mitchell MA (1990b). Hematological and morphological responses of broiler chicks to hypoxia. Avian Pathology, 19: 23-40.

McElroy JP, Harry DE, Dekkers JCM & Lamont SJ (2002). Molecular markers associated with growth and carcass traits in meat-type chickens. 7th World Congress on Genetics Applied to Livestock Production, Montpellier (France). CD-ROM communication n° 04-04.

McKay JC, Barton NF, Koerhuis ANM & McAdam J (2000). The challenge of genetic change in the broiler chicken. BSAS occasional publication n° 27: The challenge of genetic change in animal production, pp. 1-7.

McMahon CD, Popovic L, Jeanplong F, Oldham JM, Kirk SP, Osepchook CC, Wong KWY, Sharma M, Kambadur R & Bass JJ (2003). Sexual dimorphism is associated with decreased expression of processed myostatin in males. American Journal Of Physiology-Endocrinology And Metabolism, 284: E377-E381.

McMillan I & Quinton VM (2002). Selection strategies for limiting the increase in ascites while increasing growth in broilers. Poultry Science, 81: 737-744.

McPherron AC, Lawler AM & Lee SJ (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 387: 83-90.

Merat P & Ricard FH (1974). Etude d'un gène de nanisme lié au sexe chez la poule: importance de l'état d'engraissement et gain de poids chez l'adulte. In : Annales de Génétique et de Sélection Animale, 6: 211-217.

Milan D, Jeon JT, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, Rask L, Ronne H, Lundstrom K, Reinsch N, Gellin J, Kalm E, Le Roy P, Chardon P & Andersson L (2000). A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science, 288: 1248-1251.

Milan D, Leroy P, Woloszyn N, Caritez JC, Elsen JM, Sellier P & Gellin J (1995). The RN locus for meat quality maps to pig chromosome-15. Genetics Selection Evolution, 27: 195-199.

MINITAB (1998). MINITAB Reference Manual Release 12. MINITAB, Coventry.

Mitchell MA (1997). Ascites syndrome: a physiological and biochemical perspective. World's Poultry Science Journal, 53: 61-64.

Mitchell MA & Sandercock DA (1995). Creatinine kinase isoenzyme profiles in the plasma of the domestic fowl (Gallus domesticus): effect of acute heat stress. Research in Veterinary Science, 59: 30-34.

Miyake T, Dogo T, Moriya K & Sasaki Y (1999a). Bayesian analysis for existence of segregation of major genes affecting carcass traits in Japanese Black cattle population. Journal of Animal Breeding and Genetics, 116: 207-215.

Miyake T, Gaillard C, Moriya K & Sasaki Y (1999b). Accuracy of detection of major genes segregating in outbred population by Gibbs sampling using phenotypic values of quantitative traits. Journal of Animal Breeding and Genetics, 116: 281-288.

Miyake T, Moriya K & Sasaki Y (1999c). Influence of initial values on estimates by mixed inheritance model with Gibbs sampling. Journal of Animal Breeding and Genetics, 116: 39-46.

Moghadam HK, McMillan I, Chambers JR & Julian RJ (2001). Estimation of genetic parameters for ascites syndrome in broiler chickens. Poultry Science, 80: 844-848.

Morrell NW & Wilkins MR (2001). Genetic and molecular mechanisms of pulmonary hypertension. Clinical Medicine, 1: 138-145.

Morton NE & MacLean CJ (1974). Analysis of family resemblance. III. Complex segregation of quantitative traits. American Journal of Human Genetics, 26: 489-503.

Mrode RA (1996). Linear models for the prediction of animal breeding values. CAB International. 1st edition.

Neoforma Inc, 1999. URL: www.neoforma.com

Nezer C, Moreau L, Brouwers B, Coppieters W, Detilleux J, Hanset R, Karim L, Kvasz A, Leroy P & Georges M (1999). An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nature Genetics, 21: 155-156.

Olkowski AA & Classen HL (2000). Studies on the pathogenesis of hypoxemia in broiler chickens. Proceedings of the Annual Meeting of the Poultry Science Association, August 18-21, Montreal. http://www.poultryscience.org/abs00/00psab25.pdf

Olkowski AA, Korver D, Rathgeber B & Classen HL (1999). Cardiac index, oxygen delivery, and tissue oxygen extraction in slow and fast growing chickens, and in chickens with heart failure and ascites: a comparative study. Avian Pathology, 28: 137-146.

Olkowski AA, Kumor L & Classen HL (1996). Changing epidemiology of ascites in broiler chickens. Canadian Journal of Animal Science, 76: 135-140.

Ottini L, Marziali G, Conti A, Charlesworth A, Sorrentino V (1996). Alpha and beta Isoforms of ryanodine receptor from chicken skeletal muscle are the homologues of mammalian RYR1 and RYR3. Biochemical Journal, 315: 207-216.

Page BT, Casas E, Heaton MP, Cullen NG, Hyndman DL, Morris CA, Crawford AM, Wheeler TL, Koohmaraie M, Keele JW & Smith TPL (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. Journal Of Animal Science, 80: 3077-3085.

Pakdel A, Van Arendonk JAA, Vereijken ALJ & Bovenhuis H (2002a). Direct and maternal genetic effects for ascites-related traits in broilers. Poultry Science, 81: 1273-1279.

Pakdel A, Van Arendonk JAA, Vereijken ALJ & Bovenhuis H (2002b). Genetic and phenotypic correlations for ascites related traits in broilers. 7th World Congress on Genetics Applied to Livestock Production, Montpellier (France). CD-ROM communication n° 13-16.

Pavlidis HO, Balog JM, Stamps L, Huff GR, Huff WE, Rath NC, Cisar CR, Tellez G & Anthony NB (2003). Effect of hypobaric hypoxia on ascites resistant and susceptible lines and their reciprocal F_1 crosses. 1.Production parameters and ascites mortality.

Proceedings of the Annual Meeting of the Poultry Science Association, July 6-9, Monona Terrace, Madison. http://www.poultryscience.org/psa03/abstracts/abs11.pdf

Pavlidis HO, Stamps L, Hughes JDJr, Cooper MA, Balog JM, Huff WE, Huff GR, Rath NC & Anthony NB (2002). Divergent selection for ascites incidence in chickens. Proceedings of the Annual Meeting of the Poultry Science Association, August 11-14, Newark, Delaware. http://www.poultryscience.org/meet/91st/psabs13.pdf

Piper LR & Bindon BM (1982). Genetic segregation for fecundity in Boroola Merino sheep. Proceedings of the World conference on Sheep and Beef Cattle Breeding, 1:395-400.

Quane KA, Healy JMS, Keating KE, Manning BM, Couch FJ, Palmucci LM, Doriguzzi C, Fagerlund TH, Berg K, Ording H, Bendixen D, Mortier W, Linz U, Muller CR & Mccarthy TV (1993). Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. Nature Genetics, 5: 51-55.

Rabie T, Crooijmans RPMA, Vereijken A, Albers G, Veenendaal T, Dijkhof R, Poel J van der, Bovenhuis H & Groenen M (2002). Fine mapping of quantitative trait loci (QTL) affecting susceptibility to ascites in chicken. Abstracts of the 28th International Conference on Animal Genetics, Göttingen, 11-15 August, Poster E072, p. 189.

Rauw WM, Kanis E, Noordhuzien-Stassen EN & Grommers FJ (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Production Science, 56:15-33.

Rose SP (1997). Principles of Poultry Science. CAB International. 1st edition.

Ross Breeders Ltd. (1996). Producing quality broiler meat: Ross broiler management manual.

Roughton FJW (1964). Transport of oxygen and carbon dioxide. In Fenn W.O. and Rahn H. 1964. Handbook of Physiology, section 3: Respiration, volume I. American Physiological Society, Washington, D.C., 1964: 767-823

Roush WB & Wideman RF (2000). Evaluation of broiler growth velocity and acceleration in relation to pulmonary hypertension syndrome. Poultry Science, 79: 180-191.

Roush WB, Wideman RF, Cahaner A, Deeb N & Cravener TL (2001). Minimal number of chicken daily growth velocities for artificial neural network detection of pulmonary hypertension syndrome (PHS). Poultry Science, 80: 254-259.

Sandercock DA, Hunter RR, Mitchell MA & Hocking PM (2001). The effect of genetic selection for muscle yield on idiopathic myopathy in poultry: implications for welfare. In Proceedings: World's Poultry Science Association, 6th European Symposium on Poultry Welfare, Zollikofen, Switzerland. 1st-4th, September 2001.

Scheele CW (1996). Ascites in chickens: Oxygen consumption and requirement related to its occurrence. PhD Thesis, Wageningen University.

Schmid M, Nanda I, Guttenbach M, Steinlein C, Hoehn H, Schartl M, Haaf T, Weigend S, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke TA, Groenen MAM, Crooijmans RPMA, Vignal A, Fillon V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Mäki-Tanila A, Cheng HH, Delany ME, Burnside J, Mizuno S. (2000). First report on chicken genes and chromosomes 2000. Cytogenetics and Cell Genetics, 90: 169-218.

Seaton G, Haley CS, Knott SA, Kearsey M & Visscher PM (2002). QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics, 18: 339-340. http://qtl.cap.ed.ac.uk

Self SG & Liang KY (1987). Asymptotic properties of maximum-likelihood estimators and likelihood ratio tests under nonstandard conditions. Journal Of The American Statistical Association, 82: 605-610.

Sewalem A, Morrice DM, Law A, Windsor D, Haley CS, Ikeobi CON, Burt DW, & Hocking PM (2002). Mapping of quantitative trait loci for body weight at three, six, and nine weeks of age in a broiler layer cross. Poultry Science, 81: 1775-1781.

Shlosberg A, Bellaiche M, Zeitlin G, Yaacobi M & Cahaner A (1996). Hematocrit values and mortality from ascites in cold-stressed broilers from parents selected by heamatocrit. Poultry Science, 75: 1-5.

Shuster DE, Kehrli ME, Ackermann MR & Gilbert RO (1992). Identification and prevalence of a genetic-defect that causes leukocyte adhesion deficiency in holstein cattle. Proceedings Of The National Academy Of Sciences Of The United States Of America, 89: 9225-9229.

Sitsapesan R & Williams AJ (1998). The structure and function of ryanodine receptors / editors, R. Sitsapesan, A.J. Williams. Imperial College Press, London .

Smith C (1982). Estimates of trends in the halothane gene in pig stocks with selection. Zeitschrift Fur Tierzuchtung Und Zuchtungsbiologie-Journal Of Animal Breeding And Genetics, 99: 232-240.

Smith EJ, Lyons LA, Cheng HH & Suchyta SP (1997). Comparative mapping of the chicken genome using the East Lansing reference population. Poultry Science, 76: 743-747.

Sorensen DA, Andersen S, Gianola D & Korsgaard I (1995). Bayesian-inference in threshold models using Gibbs sampling. Genetics Selection Evolution, 27: 229-249.

Spelman RJ, Coppieters W, Karim L, Van Arendonk JAM & Bovenhuis (1996). Quantitative trait loci analysis for five milk production traits on chromosome six in the Dutch Holstein-Friesian population. Genetics, 144: 1799-1808.

Spiller MP, Kambadur R, Jeanplong F, Thomas M, Martyn JK, Bass JJ & Sharma M (2002). The myostatin gene is a downstream target gene of basic helix-loop-helix transcription factor MyoD. Molecular And Cellular Biology, 22: 7066-7082.

Squires EJ & Summers JD (1993). A consideration of comparative metabolic aspects of the etiology of sudden-death syndrome and ascites in broilers. British Veterinary Journal, 149: 285-294.

Stram DO & Lee JW (1994). Variance-components testing in the longitudinal mixed effects model. Biometrics, 50: 1171-1177.

Suchyta SP, Cheng HH, Burnside J & Dodgson JB (2001). Comparative mapping of chicken anchor loci orthologous to genes on human chromosomes 1, 4 and 9. Animal Genetics, 32: 12-18.

Szydlowski M & Szwaczkowski T (1998). Simulation study on the application of Gibbs sampling for major gene detection in a population of laying hens. Journal of Applied Genetics, 39: 321-330.

Szydlowski M & Szwaczkowski T (2001). Bayesian segregation analysis of production traits in two strains of laying chickens. Poultry Science, 80: 125-131.

Tatsuda K, Fujinaka K & Yamasaki T (2000). Genetic mapping of a body weight trait in chicken. Animal Science Journal, 71: 130-136.

Tiso N, Salamon M, Bagattin A, Danieli GA, Argenton F & Bortolussi M (2002). The binding of the RYR2 calcium channel to its gating protein FKBP12.6 is oppositely affected by ARVD2 and VTSIP mutations. Biochemical and Biophysical Research Communications, 299: 594-598.

Turelli M & Barton NH (1994). Genetic and statistical-analyses of strong selection on polygenic traits: What, me normal? Genetics, 138: 913-941.

Utz HF, Melchinger AE & Schön CC (2000). Bias and sampling error of the proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics, 154: 1839-1849.

Van der Beek S & Van Arendonk JAM (1996). Marker-assisted selection in an outbred poultry breeding nucleus. Animal Science, 62: 171-180.

Van Horne P (2000). Broiler production costs across Europe: new pressures hitting all countries. Cobb Quarterly review. URL: www.cobb-vantress.com.

Van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ & Van Arendonk JAM (1999). Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. Poultry Science, 78: 15-23.

Van Kaam JBCHM, Van Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, Van der Poel JJ & Veenendaal A (1998). Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. Livestock Production Science, 54: 133-150.

Visscher PM, Thompson R & Haley CS (1996). Confidence intervals in QTL mapping by bootstrapping. Genetics, 143: 1013-1020.

Walling GA, Bishop SC, Pong-Wong R, Gittus G, Russel AJF & Rhind SM (2002). Detection of a major gene for litter size in Thoka Cheviot sheep using Bayesian segregation analyses. Animal Science, 75: 339-347.

Weller JI, Kashi Y & Soller M (1990). Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy-cattle. Journal Of Dairy Science, 73: 2525-2537.

Wideman RF & French H (1999). Broiler breeder survivors of chronic unilateral pulmonary artery occlusion produce progeny resistant to pulmonary hypertension syndrome (ascites) induced by cool temperatures. Poultry Science, 78: 404-411.

Wideman RF & French H (2000). Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. Poultry Science, 79: 396-401.

Wideman RF, Wing T, Kirby YK, Forman MF, Marson N, Tackett CD & Ruiz-Feria CA (1998). Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. Poultry Science, 77: 1565-1573.

Yonash N, Bacon LD, Witter RL & Cheng HH (1999). High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Animal Genetics, 30: 126-135.

Zeng Z-B (1993). Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proceedings of the National Academy of Sciences (USA), 90: 10972-10976.

Zhao L, Sebkhi A, Nunez DJR, Long L, Haley CS, Szpirer J, Szpirer C, Williams AJ & Wilkins MR (2001). Right ventricular hypertrophy secondary to pulmonary hypertension is linked to rat chromosome 17 - Evaluation of cardiac ryanodine RYR2 receptor as a candidate. Circulation, 103: 442-447.